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MEMORANDUM

SUBJECT: ***AZINPHOS-METHYL*** - Report of the Hazard Identification Assessment Review Committee.

FROM:
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and
Jess Rowland
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Health Effects Division (7509C)

THROUGH:
K. Clark Swentzel, Chairman,
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)
And
Mike Metzger, Co-Chairman
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

TO:
Alberto Protzel, Branch Senior Scientist
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PC Code: 058001

On March 19, 1998, the Health Effects Division's Hazard Identification Assessment Review committee evaluated the toxicology data base of azinphos-methyl to re-assess the Reference Dose and select the toxicological endpoints and dose levels for acute dietary as well as occupational and residential risk assessments. The Committee also addressed the potential sensitivity of infants and children as required by the Food Quality Protection Act (FQPA) of 1996. The Committee's conclusions are presented in this report.

Committee Members in Attendance

Members in attendance were: Karl Baetcke, William Burnam, Robert Fricke, Nancy McCarroll, Mike Metzger, Jess Rowland (Executive Secretary) and Clark Swentzel (Chairman). Member in absentia: Karen Hamernik, Susan Makris and Melba Morrow.

Other HED members also present were: Mike Ioannou and Alberto Protzel of Toxicology Branch 1, Kathy Raffaele of Toxicology Branch 2, Catherine Eiden of Risk Characterization & Analysis Branch and Jack Authur, Chemistry & Exposure Branch 2. Data was presented by Nancy McCarroll of Toxicology Branch 1.

Data Presentation: and Report Preparation	Nancy McCarroll Toxicologist
Report Concurrence:	Jess Rowland Executive Secretary

I. INTRODUCTION

On September 16, 1993, the Health Effect's Division's RfD/Peer Review Committee established a Reference Dose of 0.00149 mg/kg/day based on a NOEL of 0.149 mg/kg/day established in a chronic toxicity study in dogs and an Uncertainty Factor of 100 for inter-species extrapolation and intra-species variation (*Memorandum:* G. Ghali, HED to L. Rossi, RD, Dated 12/07/93).

On February 27, 1997, the Health Effects Division's Toxicology Endpoint Selection (TES) Committee selected the doses and endpoints for acute dietary as well as occupational and residential exposure risk assessments. The TES Committee did not address the FQPA requirement because of the pending Agency's assessment of organophosphates and their neurotoxic potential (TES Document, 2/27/97).

On December 10, 1997, the Health Effects Division's Hazard Identification Assessment Review Committee (HIARC) met to re-evaluate the Uncertainty Factors and MOEs for dietary as well as non-dietary risk assessments. This re-evaluation was necessary to ensure consistency with the other organophosphate chemicals that were recently reviewed by the HIARC to address the enhanced sensitivity of infants and children as required by the FQPA. At the meeting, the Committee evaluated the toxicology data base and determined that a reexamination of the subchronic neurotoxicity study in rats, the neuropathology findings from the chronic feeding/carcinogenicity study in rats and the neuropathology data from the one-year dog study should be performed. In addition, a search of the open literature was recommended. These actions were requested to determine whether a developmental neurotoxicity study with Azinphos-methyl is needed.

On March 19, 1998, the Health Effects Division's Hazard Identification Assessment Review committee evaluated the toxicology data base of azinphos-methyl to re-assess the Reference Dose and determine the Uncertainty Factor and/or Margins of Exposure for dietary and non-dietary exposure risk assessments. The Committee also addressed the potential sensitivity of infants and children as required by the Food Quality Protection Act (FQPA) of 1996. The application of the FQPA safety factor for the protection of infants and children as required by FQPA, will be determined during risk characterization.

The conclusions of the March 19, 1998 HIARC meeting, which included a determination of the Uncertainty Factors and/or the Margins of Exposure for exposure scenarios (acute and chronic dietary as well as occupational/residential risk assessments), recommendations made for aggregate exposure risk assessments and the determination of the potential susceptibility to infants and children, are presented in this report.

This report supersedes the previous RfD and TES Committee reports.

II. HAZARD IDENTIFICATION

A. Acute Dietary Risk Assessment (Acute RfD)

Study Selected: Acute Neurotoxicity - Rat §81-8

MRID No. 43360301

Executive Summary: In an acute neurotoxicity study, male and female Fischer 344 rats (18/sex/dose) received a single oral administration of azinphos-methyl (92.2%) at 0, 2, 6, or 12 mg/kg (males) or 0, 1, 3, or 6 mg/kg (females). Mortality occurred in 5 males and 15 females at the high dose. An increased incidence of neurobehavioral effects was seen in males at 6 mg/kg and in females at 3 mg/kg. The neurobehavioral signs included gait incoordination, repetitive chewing, muscle fasciculations, tremors, hypoactivity, no reaction to touch, abnormal righting reflex, decreased body temperature, decreased forelimb and/or hind limb grip strength, and decreased motor and locomotor activities. Statistically significant inhibition of cholinesterase (ChE) activity was observed in both sexes at all dose levels on Day 0 (90 minutes post dosing). When compared to controls, decreases in males were -32% for plasma ($p < 0.05$), -33% for RBC ($p < 0.05$) and -15% for brain; decreases in females were -11% for plasma, -17% for RBC ($p < 0.05$) and -5% for brain.

During the evaluation of this study, the Committee determined that the inhibition of ChE activity for all three biomarkers (plasma, RBC and brain) observed at the lowest dose tested in males (2 mg/kg/day) and females (1 mg/kg/day) was "biologically" significant and, therefore, was attributed to treatment. The Data Evaluation Record (DER) established NOELs of 2 mg/kg for males and 1 mg/kg for females and LOELs of 6 mg/kg for males and 3 mg/kg for females. Based on the inhibition of ChE activity, the Committee concluded that a NOEL was not established in this study and the LOEL was 1 mg/kg.

Dose and Endpoint for Risk Assessment: LOEL = 1 mg/kg, based on plasma, RBC and brain ChE; a NOEL was not established.

Uncertainty Factor (UF): 300 (10x for inter-species extrapolation, 10x for intra-species variability and 3x for the lack of a NOEL in the critical study).

$$\textbf{Acute RfD} = \frac{1.0 \text{ mg/kg}}{300 \text{ (UF)}} = \textbf{0.003 mg/kg}$$

Comments about Endpoint and/or Study: This study was selected for establishing the acute RfD because the critical effect (brain, erythrocyte and plasma cholinesterase inhibition) was seen following a single exposure. An additional UF was included because of the use of a LOEL (i.e., lack of a NOEL in the study) for this risk assessment.

This risk assessment is required.

B. Chronic Dietary [Reference Dose (RfD)]

Study Selected: One-year Toxicity Study in Dogs §83-1b

MRID No. 41804801

Executive Summary: In a 52-week toxicity study, azinphos-methyl technical (91.9%) was administered to male and female beagle dogs (4/sex/group) at dose levels of 0, 5, 25 or 125 ppm (0.149, 0.688 or 3.844 mg/kg for males; 0.157, 0.775 or 4.333 mg/kg for females). Both sexes of dogs at 125 ppm exhibited decreases in plasma ChE (52-58%), RBC ChE (66-92%) and brain ChE (20-27%) beginning at week 4 of treatment and continuing until week 52. At the 125 ppm dose level, cytochrome P-450 N- and O-demethylase activity was increased 39% in male dogs. Serum albumin and the A/G (albumin to globulin) ratio was reduced by 13 and 20%, respectively, in the male dogs after 13 weeks of exposure. Mucoid diarrhea and occasional emesis were also observed at this dose level in male and female dogs. At 25 ppm, RBC ChE activity was decreased by 27-40% in male dogs, and by 35-43% in female dogs. An increased incidence of mucoid diarrhea was also observed. The **NOEL was 0.149 mg/kg/day for males and 0.157 mg/kg/day for females,** and the **LOEL was 0.688 mg/kg/day for males and 0.775 mg/kg/day for females,** based on the above noted significant decreases in RBC ChE activity in both sexes as well as an increased incidence of diarrhea in males.

Dose and Endpoint for Establishing the Chronic RfD: NOEL = 0.149 mg/kg/day, based on RBC ChE inhibition at 0.688 mg/kg/day (LOEL).

Uncertainty Factor (UF): 100 (10x for inter-species extrapolation and 10x for intra-species variability)

$$\textbf{Chronic RfD} = \frac{0.149 \text{ mg/kg/day}}{100 \text{ (UF)}} = \textbf{0.0015 mg/kg/day}$$

Comments about Study and Endpoint: The HIARC concurred with the dose, endpoint and the Uncertainty Factor selected by the RfD Committee in 1993 in deriving the chronic RfD.

C. Occupational/Residential Exposure

1. Dermal Absorption

Study: Dermal Absorption Study in Rats §85-2

MRID No. 42452701

Executive Summary: In a dermal absorption study, azinphos-methyl (as Guthion 35 Wettable Powder) was applied to six groups of four male Sprague Dawley rats/group at doses of 0, 0.93, 9.3 or 93 $\mu g/cm^2$ (equivalent to 0.056, 0.56, or 5.6 mg/kg) active ingredient for exposure durations of 1, 4, 10, 24, 72 and 168 hours. Exposure sites for animals in the 24, 72 and 168 hour exposure groups were washed at 10 hours. Plasma and erythrocyte cholinesterase was determined in treated and control group animals. At 10 hours, 32.24, 22.08 and 23.71% of the dose remained on the washed skin and continued to be absorbed throughout the subsequent exposure period. Maximum systemic absorption was 41.65, 21.86 and 18.34% at 168 hours for the respective dose groups (see Table below). In the high-dose group, RBC ChE inhibition at 10-24 hours was significantly lower (by 16 to 17%) than control. No effects on plasma ChE were note at any dose and no effects on RBC ChE were seen at levels ≤0.56 mg/kg. Based on the inhibition of RBC ChE, the NOEL and LOEL are 0.56 and 5.6 mg/kg, respectively.

	Percent of Total Dose Absorbed		
Average Dose	1 Hour	10 Hours	10-168 Hours[a]
0.056 mg/kg	9.41	22.71	41.65
0.56 mg/kg	3.67	15.16	21.86
5.6 mg/kg	0.48	2.86	18.34

[a] Test sites were washed at 10 hours.

Dermal Absorption Factor: Based on the above findings, the dermal absorption factor is 41.65%.

2. Short-Term Dermal - (1-7 days)

Study Selected: Dermal Absorption Study in Rats §85-2

MRID No. 42452701

Executive Summary: See Dermal Absorption

Dose and Endpoint Selected for the Short-Term Dermal Risk Assessment: NOEL = 0.56 mg/kg, based on RBC ChE inhibition at 5.6 mg/kg (LOEL).

Comments about Study and Endpoint: The TES Committee selected the 21-day dermal toxicity study in rabbits (MRID No. 00145715) for the Short- and Intermediate-Term Occupational or Residential Exposure Risk Assessments. However, during the evaluation of the data base for azinphos-methyl, the HIARC determined that the 21-day dermal toxicity study in rabbits was not appropriate for the following reasons:

- The validity of rabbit dermal exposure studies for organophosphate pesticides which are activated via oxidative desufuration including azinphos-methyl, has been questioned based on evidence indicating that the rabbit has an enhanced ability to detoxify these organophosphate pesticides that are administered dermally as compared to other species.
- The comparative analysis of data from acute dermal testing in rabbits (MRID No. 40280102) and rats (MRID No. 00155003) indicated an ≈10-fold difference in the LD_{50} values (i.e., the LD_{50} for rabbits was >2000 mg/kg for both sexes while the LD_{50} for rats was 200-250 mg/kg ♂ and 155 mg/kg ♀) . This finding suggests that using data from dermal studies performed with rabbits may underestimate the risk.

- Assuming that rabbits are less sensitive to the toxic effects of azinphos-methyl via the dermal route and that the proportionality observed in the acute rat and rabbit dermal studies would persist, it is plausible that a NOEL for rats in a 21-day dermal study would be appreciable lower than the NOEL (2.0 mg/kg/day) established for rabbits.

  Based on the above considerations (i.e., the rat toxicity data maybe more protective than the rabbit data), the HIARC determined that the dermal absorption study in rats (MRID No. 42452701), which included a determination of ChE inhibition, is appropriate for the Short-Term Occupational or Residential Exposure Risk Assessment.

This risk assessment is required.

3. Intermediate-Term Dermal (7 Days to Several Months)

Study Selected: One Year Toxicity Study - Dog §83-1b

MRID No.: 41804001

Summary: See Chronic Dietary

Dose and Endpoint for Risk Assessment: NOEL = 0.149 mg/kg/day based on RBC ChE inhibition observed at the 4-week measurement in male dogs at 0.688 mg/kg/day (LOEL).

Comments about Endpoint and/or Study: The 21-day rabbit study was also selected by the TES Committee for the Intermediate-Term Occupational or Residential Exposure Risk Assessment. For reasons similar to those outlined above (See above commentary), the HIARC selected the one year toxicity study in dogs for this Exposure Risk Assessment. **Since an oral NOEL was selected a dermal absorption factor of 41.65% should be used for this risk assessment.** Application of the dermal absorption factor (0.42) to the above NOEL yields an equivalent dermal dose of 0.36 mg/kg/day.

This risk assessment is required.

4. Long-Term Dermal (Several Months to Life-Time)

Study Selected: None

MRID No. None

Executive Summary: None

Dose/Endpoint for Risk Assessment: Not Applicable

Comments about Study and Endpoint: Based on the current use pattern, Long-Term exposure via the dermal route is not expected.

This risk assessment is not required.

5. Inhalation Exposure (Any-Time period)

Study Selected.: 90-Day Inhalation Study-Rat §82-4

MRID No.: 00155011

Executive Summary: In a subchronic inhalation toxicity study, male and female Wistar rats were exposed (via inhalation) to azinphos-methyl aerosol concentrations of 0.195, 1.24 or 4.72 mg/m^3 (equivalent to 0.0002, 0.0012 and 0.0047 mg/L, respectively), 6 hours/day, 5 days/week for 90 days. Plasma and RBC ChE activity was determined after 2, 4, 6, 8 and 12 weeks of treatment. Plasma and RBC ChE inhibition (30-40%) was observed in both sexes at a concentration of 0.0047 mg/L. The NOEL was determined to be 0.0012 mg/L and the LOEL was determined to be 0.0047 mg/L.

Dose and Endpoint for Use in Risk Assessment: NOEL = 0.0012 mg/L, based on plasma and RBC ChE inhibition at 0.0047 mg/L (LOEL).

Comments about the Endpoint and/or Study: Since this is the only inhalation study available, this NOEL should be used for performing the short-term, intermediate-term and chronic inhalation risk assessments.

This risk assessment is required.

D. Margin of Exposure for Occupational/Residential Exposures:

There are no registered residential uses of azinphos-methyl at the present time. For Short- and Intermediate-Term dermal occupational exposure risk assessments and inhalation occupational exposure risk assessments, the HIARC recommends **a Margin of Exposure (MOE) of 100.**

E. Recommendation for Aggregate Exposure Risk Assessments

For the aggregate exposure risk assessment, the MOE's derived for the oral, dermal and inhalation exposures may be combined to obtain a total MOE since a common toxicological endpoint (i.e., cholinesterase inhibition) was observed in oral, dermal and inhalation toxicity studies/routes.

$$\text{Total MOE}_t \quad \frac{1}{\text{MOE}_{\text{Oral}}} + \frac{1}{\text{MOE}_{\text{Dermal}}} + \frac{1}{\text{MOE}_{\text{Inhalation}}}$$

III. CLASSIFICATION OF CARCINOGENIC POTENTIAL

At the September 1993, meeting of the RfD/Peer Review Committee, azinphos-methyl was classified as a **"not likely"** human carcinogen. This classification was based on the lack of evidence of carcinogenicity in male and female CD-1 mice (MRID No. 00147895) and in male and female Wistar rats (MRID No. 41119901). In both studies, the highest dose tested was considered adequate for carcinogenicity testing based on cholinesterase inhibition. Treatment with azinphos-methyl did not alter the tumor profile in the above strain of mice or rats. The HIARC concurred with these conclusions and re-affirmed the previous classification.

IV. FQPA CONSIDERATIONS

1. Neurotoxicity Data

(i) Hen

In an acute delayed neurotoxicity study in hens, azinphos-methyl (85%) was administered by gavage at 330 mg/kg (LD_{50}). A second dose (330 mg/kg) was given by gavage at study day 21. Mortality was extensive (18/30 hens died within 3-4 days of the initial dose and one additional hen died following the second dose), and clinical signs of neurotoxicity were observed (grade 5 ataxia, prostration, hypoactivity, liquid feces). According to the DER, no gross or microscopic evidence of neuropathology (nonperfused tissues) was observed. neurotoxic esterase (NTE) was not apparently measured. The Committee noted the observations of degenerative changes (i.e., digestion chambers and perivascular cuffing in the brain) of the nervous system of the treated animals but concluded that the effects were not treatment-related (MRID No. 40883101).

Based upon the conclusion that there were negative findings of neuropathology in the acute delayed neurotoxicity study in hens, a subchronic delayed neurotoxicity study in hens was not submitted to the Agency

(ii) Rats

In an acute neurotoxicity study, male and female Fischer 344 rats (18/sex/dose) received a single oral administration of azinphos-methyl (92.2%) at 0, 2, 6, or 12 mg/kg (males) or 0, 1, 3, or 6 mg/kg (females). Mortality occurred in 5 males and 15 females at the high dose. An increased incidence of neurobehavioral effects was seen in males at 6 mg/kg and in females at 3 mg/kg. The neurobehavioral signs included gait incoordination, repetitive chewing, muscle fasciculations, tremors, hypoactivity, no reaction to touch, abnormal righting reflex, decreased body temperature, decreased forelimb and/or hind limb grip strength, and decreased motor and locomotor activities. Statistically significant inhibition of cholinesterase activity was observed in both sexes at all dose levels on Day 0 (90 minutes post dosing). When compared to controls, decreases in males were -32% for plasma ($p < 0.05$), -33% for RBC ($p < 0.05$) and -15% for brain; decreases in females were -11% for plasma, -17% for RBC ($p < 0.05$) and -5% for brain (MRID No. 43360301).

During the evaluation of this study, the Committee determined that the inhibition of cholinesterase activity for all three biomarkers (plasma, RBC and brain) observed at the lowest dose tested in males (2 mg/kg/day) and females (1 mg/kg/day) was "biologically" significant and therefore was attributed to treatment. The Data Evaluation Record (DER) established NOELs of 2 mg/kg for males and 1 mg/kg for females and LOELs of 6 mg/kg for males and 3 mg/kg for females . Based on the inhibition of cholinesterase activity, the Committee concluded that a NOEL was not established in this study and the LOEL was 1 mg/kg (MRID No. 43360301).

In a subchronic neurotoxicity study, groups of Fischer 344 rats (18/sex/dose) received azinphos-methyl (92.2%) in the diet at dose levels of 0, 15, 45, 90 (males and females) or 120 ppm (males) for 13 weeks. These dose levels were equivalent to 0, 0.91, 2.81 or 7.87 mg/kg/day for males and 0, 1.05, 3.23 or 6.99 mg/kg/day for females. Treatment-related effects included: decreases in body weight and body weight gain (both sexes at the high-dose); cholinergic signs including increased reactivity, uncoordinated gait and tremors (both sexes at mid and/or high doses); significant inhibition of plasma and brain (both sexes mid and high doses) and RBC (all doses) cholinesterase activity; decreased forelimb grip strength, motor activity and locomotor activity (both sexes high dose); and a possible increase in histopathological lesions in the spinal cord, brain, optic nerve (both sexes at high dose) (MRID No. 43826601).

The neuropathology findings were equivocal, but suggested treatment-related effects in the brain (axonal swelling of minimum severity in males) and spinal cord (nerve fiber degeneration of the cauda quina and the cervical and/or thoracic cord in both sexes) at the high dose (120 ppm; 7.87/6.99 mg/kg/day in M/F). In females, the DER noted a possible correlation between the incidence of cervical spinal cord lesions at the high dose and decreased forelimb grip strength at all dose levels. Since the histopathology tables were not

included in the DER, the Committee recommended that the incidence and severity of the equivocal neuropathological findings be reassessed. Based on a reevaluation of the neuropathology data (see Memo dated March 19, 1998), it was concluded that neither the incidence nor the severity of the neuropathological lesions noted in high-dose males and females could be attributed to treatment with azinphos-methyl. The findings were not statistically significant, of minimal severity and occurred sporadically.

(iii) Other Relevant Neurotoxicity Findings

In addition to the clinical signs of neurotoxicity which were observed in the neurotoxicity studies in rats, the following additional clinical observations that are indicative of neurotoxicity were seen: occasional emesis and mucoid diarrhea at 125 ppm (0.688 mg/kg/day) in the 1-year dog study, convulsions at 2.25 mg/kg/day in the two-generation reproduction study in rats, and tremors at 6 mg/kg/day in the prenatal developmental toxicity study in rabbits. Similarly, ChE inhibition (plasma, RBC, and brain) was observed at low dose levels in all subchronic and chronic studies in which this parameter was measured.

In contrast, there was no indication of decreased brain weight or histopathology of the brain or peripheral nervous system, following processing of tissues without perfusion, in any of the guideline subchronic or chronic studies. The Committee, however, noted that numerous neurological tissues were apparently not assessed in the chronic dog study (MRID No. 41804801) and that histopathology tables were not provided in the DER of the chronic rat study (MRID No. 41119901).

However, a reexamination of the neuropathology data presented in the one-year dog study (see Memo dated March 19, 1998) indicated that no lesion were found in the brain, spinal cord, eyes, optic nerve or sciatic nerve. Samples of the above tissues were processed and examined microscopically for all animals in all study groups.

Similarly, a reevaluation of the neuropathology data from the chronic rat study (see Memo dated March 19, 1998) revealed that neither the peripheral nerve nor the spinal cord were examined histologically. Although this study is currently classified as Acceptable, it does not fully satisfy the guideline requirements for a chronic feeding/ carcinogenicity study (83-1) in rats. However, it was not chosen as a critical study for the toxicity endpoint selection. In addition, reassessment of the brain weight data in this study, indicated that significantly increased relative brain weights in males of the mid-(15 ppm) and high-(45 ppm) dose groups at 12 months and in high-dose males at 24 months were accompanied by significant body weight reductions. However, absolute brain weights for these groups showed nonsignificant less than or equal to 3% increases. It was concluded, therefore, that the apparent increase in relative brain weights was an artifact resulting from decreased body weight.

The reevaluation of the data related to neurological findings indicates that while azinphos-methyl is a potent cholinesterase inhibitor, there is no evidence in the submitted studies or the open literature that demonstrate an association between exposure to the test chemical and histopathological effects on the nervous system of either the rat or the dog.

2. Developmental Toxicity

In a prenatal developmental toxicity study, pregnant Crl:CDBR rats (33/dose) received oral administration of azinphos-methyl (87.7%) in aqueous Emulphor-719 at doses of 0, 0.5, 1.0 or 2 mg/kg/day during gestation days 6 through 15. Fetal brain cholinesterase activity was measured on Day 20 of gestation. For maternal toxicity, the NOEL was 0.5 mg/kg/day and the LOEL was 1 mg/kg/day based on inhibition of red blood cell and brain cholinesterase activity. No developmental toxicity was observed. A significant decrease in fetal brain cholinesterase activity on gestation day 20 at 0.5 mg/kg/day was not considered to be toxicologically significant since it was only seen in the low dose animals. For developmental toxicity, the NOEL was >2 mg/kg/ day (HDT) (MRID No. 40464801).

In a prenatal developmental toxicity study, pregnant American Dutch rabbits (20/dose) received oral administration of Azinphos-methyl (87.7%) in aqueous Emulphor (7%) at doses of 0, 1.0, 2.5 or 6.0 mg/kg/ day during gestation days 6 through 18. Cholinesterase activity was not measured in pups. For maternal toxicity, the NOEL was 1 mg/kg/day and the LOEL was 2.5 mg/kg/day based on significant inhibition of plasma and red blood cell cholinesterase activity on gestation Day 19. On gestation Day 28, only brain cholinesterase was decreased at 6 mg/kg/day; clinical cholinergic signs (tremors) were also seen at 6 mg/kg/day. For developmental toxicity, the NOEL was 2.5 mg/kg/day and the LOEL was 6 mg/kg/day based on increases in pre-and post-implantation loss (MRID No. 40713901).

3. Reproductive Toxicity

In a one-generation reproduction study, Wistar rats were fed diets containing azinphos-methyl (92%) at 0, 5, 15 or 45 ppm (0, 0.55. 1.54 or 4.87 mg/kg/day, respectively). There was no increased sensitivity of pups over the adults. The parental systemic LOEL was 5 ppm (0.55 mg/kg/day) based on significant inhibition of plasma and erythrocyte cholinesterase activity on Day 5 of lactation ; a parental systemic NOEL was not established. Further characterization of maternal cholinesterase inhibition revealed that plasma, RBC, and brain cholinesterase activity were significantly decreased in females at 45 ppm at all time points tested (end of premating, gestation Day 11, lactation Day 5 and lactation Day 28). At 15 ppm, plasma and RBC (not brain) ChE were significantly inhibited at the same time points. For males at the end of mating, plasma ChE was significantly decreased at 15 and 45 ppm, while RBC ChE was significantly decreased at 5, 15, and 45 ppm; brain ChE was not decreased at any dietary level. For offspring toxicity, the NOEL was 15 ppm (1.54 mg/kg/day) and the LOEL was 5 ppm (0.55 mg/kg /day) based on significant decreases

in pup viability index (death of the offspring during postnatal days 0-5) and decreased pup weights at postnatal Days 14 and 21. Pup brain weight and cholinesterase activity were assessed in pups at postnatal Days 5 and 28. At 45 ppm, significant inhibition of brain cholinesterase activity was seen on Days 5 and 28, and a significant reduction in brain weight was seen on post natal Day 5, but not Day 28. This study was submitted as supplementary data to the two-generation study (discussed below) (MRID No. 41916801).

In a two-generation reproduction study, Wistar rats were fed diets containing azinphos-methyl (87.2%) at 0, 5, 15 or 45 ppm (0, 0.25, 0.75, or 2.25 mg/kg/day, respectively) for two successive generations. For parental systemic toxicity, the NOEL was 15 ppm (0.75 mg/kg/day and the LOEL was 45 ppm (2.25 mg/kg/day) based on mortality, decreased body weight for P males and F_1 males and females and clinical signs of toxicity including poor coordination and convulsions. For offspring toxicity, the NOEL was 5 ppm (0.25 mg/kg/day) and the LOEL was 15 ppm (0.75 mg/kg /day) based on a reductions in pup viability and lactation indices (death of the offspring between the time periods of postnatal days 0-5 and 5-28) and decreased mean total litter weights at weaning on postnatal Day 28. Cholinesterase activity was not measured either in the parental animals or pups (MRID No. 40332601).

4. Additional Information from the Literature

A search of the open literature from 1969 to the present revealed no increased susceptibility in rats or mice exposed *in utero* to azinphos-methyl (Short et al., 1980); there was also no evidence of neuropathology in mice receiving azinphos-methyl in drinking water for 14 weeks (Bali et al., 1996). No studies were found in the open literature regarding potential adverse effects associated with humans accidentally or occupationally exposed to azinphos-methyl.

5. Determination of Susceptibility

The developmental toxicity studies in rats and rabbits showed no evidence of additional sensitivity of young rats or rabbits following *in utero* exposure to azinphos-methyl. In the prenatal developmental toxicity study in rats, no evidence of developmental toxicity was seen even in the presence of maternal toxicity (cholinesterase inhibition).

In the two-generation reproduction study in rats, however, there was a suggestion of increased sensitivity to the offspring following pre-and/or postnatal exposure to azinphos-methyl. In both the one- and two-generation studies, decreased pup survival in both early and late stages of lactation and pup weight reductions in late lactation were observed. In the two-generation study, these effects in the offspring were observed at a dietary level which was not systemically toxic to the parental animals. It was noted, however, that parental toxicity in the one-generation study was based upon decreased cholinesterase activity, while cholinesterase measurements were not conducted in the two-generation study, and the

parental toxicity was based upon mortality, clinical signs, and body weight decrements (less sensitive indicators). **The HIARC, therefore, concluded that the suggested susceptibility of the offspring was an artifact of the study design.**

Comparative cholinesterase inhibition data for adult rats and their fetuses or pups did not identify increased susceptibility to the offspring. In the prenatal developmental toxicity study in rats, brain cholinesterase activity did not appear to be significantly inhibited in GD20 rat fetuses following *in utero* exposure, even at a dose which demonstrated marked brain cholinesterase inhibition in the dams on the same day of gestation. Brain cholinesterase inhibition in 5- and 28-day old pups of the one-generation reproduction study occurred at the highest dietary level tested; however, brain cholinesterase inhibition was also observed in maternal animals at this dose level at termination.

5. Developmental Neurotoxicity

At the RfD Peer Review Committee meeting on September 16, 1993, it was recommended that a developmental neurotoxicity study in rats be conducted with azinphos-methyl because it is a potent cholinesterase inhibitor. In retrospect, the following additional information was considered by the HIARC:

(i). Evidence that support requiring a developmental neurotoxicity study:

- SAR concern: Azinphos-methyl is an organophosphate.

- Administration to various species (rat, mouse, dog) results in cholinesterase inhibition in the plasma, erythrocytes and/or brain. Systemic evidence of cholinergic effects occurs regularly in the data. Guideline neurotoxicity studies have been submitted and demonstrate neurobehavioral effects.

- In a one-generation reproduction study in rats, dietary administration of azinphos methyl (HDT) to parental animals resulted in a significant decrease in pup brain weight on postnatal Day 5 but not Day 28.

(ii). Evidence that do not support requiring a developmental neurotoxicity study:

- With the exception cited above of decreased pup brain weight in the one-generation reproduction study, no effects on brain weight or histopathology of the brain or peripheral system (without perfusion) were observed in any of the guideline subchronic or chronic studies in which these parameters were measured.

- No evidence of abnormalities in the development of the fetal nervous system were observed in the prenatal developmental toxicity studies in either rats or rabbits at maternally toxic oral doses up to 2.0 or 6.0 mg/kg/day, respectively.

- A search of the open literature from 1969 to the present revealed no evidence of neuropathology in treated animals. No studies were found in the open literature regarding potential adverse effects associated with humans accidentally or occupationally exposed to azinphos-methyl.

- Azinphos-methyl did not cause delayed neurotoxicity in hens following acute exposure.

Based on the weight-of-the-evidence, the HIARC determined that a developmental neurotoxicity study is **not required.**

6. Determination of the FQPA Safety Factor:

The application of a FQPA factor to ensure the protection of infants and children from exposure to azinphos-methyl, as required by FQPA, will be determined by the FQPA Safety Factor Assessment Review Committee.

The HIARC, based on the hazard assessment, recommends to the FQPA Safety Committee, that the additional 10 x factor should be removed because:

(i) Developmental toxicity studies showed no increased sensitivity in fetuses as compared to maternal animals following *in utero* exposure in rats and rabbits.

(ii) Both a one- and a two-generation reproductive toxicity study in rats showed no increased susceptibility in pups when compared to adults.

(iii) There was no evidence of abnormalities in the development of the fetal nervous system in the pre/postnatal studies. Neither brain weight nor histopathology (nonperfused) of the nervous system was affected in the subchronic and chronic toxicity studies.

(iv) The toxicology data base is complete and there are no data gaps. There is no evidence to require a developmental neurotoxicity study.

The final recommendation on the FQPA Safety Factor, however, will be made during risk characterization by the FQPA Safety Committee.

V. DATA GAPS

There are no data gaps for the standard Subdivision F Guideline requirement for a food-use chemical by 40 CFR Part 158.

The HIARC did not recommend a developmental neurotoxicity study, therefore, there are no significant uncertainties in the assessment of functional development following pre- and/or postnatal exposure to azinphos-methyl.

VI. HAZARD CHARACTERIZATION

Azinphos-methyl is an organophosphate pesticide. The toxicology data base provides overwhelming evidence confirming that azinphos-methyl has anticholinesterase activity in various species including dogs, rabbits, rats, mice and hens. In acute toxicity studies, azinphos-methyl exhibits low to high toxicity depending on the route of administration and the species used. It is acutely toxic at relatively low oral or dermal doses when tested in rats but found to have low toxicity in rabbits exposed dermally. This finding supports the earlier arguments regarding the suitability of conducting rabbit dermal studies on organophosphates (see Short-Term Dermal Risk Assessment). The data from the only available acute inhalation study suggest that azinphos-methyl is moderately toxic via this route. It is only slightly irritating to the eye and non-irritating to the skin but did produce dermal sensitization in guinea pigs. Other toxic signs observed in animals treated acutely with azinphos-methyl are consistent with cholinesterase inhibition and are typical of the acute toxic signs induced by the organophosphate class of chemicals. They included: tremors, convulsions salivation, and dyspnea (labored breathing). Dose-related inhibition of plasma, erythrocyte and brain cholinesterase (ChE) activity occurs by all routes of exposure and following exposure for various durations. Although frank neurobehavioral observations have been noted in acute and subchronic studies, there is no evidence of histopathological effects on the central nervous system. Similarly, azinphos-methyl did not cause delayed neurotoxicity in hens and there was no evidence of neuropathology in chronic studies. There is also no indication of an increased sensitivity of the offspring of rats or rabbits after pre-natal and/or postnatal exposure to azinphos-methyl. In all studies examined, maternal or parental NOELs are lower or equivalent to the offspring NOELs. **Based on these considerations, the weight-of-the-evidence evaluation of the data base does not indicate the need for evaluation of functional development and, thus, there does not appear to be a need to conduct a developmental neurotoxicity study.** Azinphos-methyl has been classified in **"Group E"** (i.e., the chemical is characterized as **"Not Likely"** to be carcinogenic in humans via relevant routes of exposure) because there is no evidence that azinphos-methyl altered the spontaneous tumor profile in rats or mice. In both studies, the highest dose tested was considered adequate for carcinogenicity testing based on cholinesterase inhibition. Similarly, there is no mutagenicity concerns.

Azinphos-methyl is degraded and/or eliminated within 72 hours postdosing and does not accumulate in tissues. The metabolism of azinphos-methyl in rats proceeds largely through the action of glutathione-S-transferase and mixed function oxidases. There were no major sex- or dose-related differences in the disposition or metabolism of azinphos-methyl.

VII. ACUTE TOXICITY

Acute Toxicity of Azinphos-methyl

Guideline No.	Study Type	MRID #(S).	Results	Toxicity Category
81-1	Acute Oral (Rat)	00155002	LD_{50} =4.6 mg/kg♂ 4.4 mg/kg♀	I
81-2	Acute Dermal (Rabbit)	40280102	LD_{50} = >2000 mg/kg	III
81-2	Acute Dermal (Rat)	00155003	LD_{50} =200-250 mg/kg♂ 155 mg/kg♀	I
81-3	Acute Inhalation (Rat)	40280103	LC_{50} = >0.21mg/L	II
81-4	Primary Eye Irritation (Rabbit)	43337501	No ocular effects at 48 hrs.	III
81-5	Primary Skin Irritation (Rabbit)	43337101	Non-irritating	IV
81-6	Dermal Sensitization (Guinea Pig)	41064401	Sensitizer	N/A

VIII. SUMMARY OF TOXICOLOGY ENDPOINT SELECTION

The doses and toxicological endpoints selected for various exposure scenarios are summarized below.

EXPOSURE SCENARIO	DOSE (mg/kg/day)	ENDPOINT	STUDY
Acute Dietary	LOEL = 1.0	Plasma, erythrocyte and brain cholinesterase inhibition	Acute Neurotoxicity-Rat
	UF= 300	**Acute RfD = 0.003 mg/kg**	
Chronic Dietary	NOEL= 0.149	Erythrocyte cholinesterase inhibition.	1-Year Toxicity- Dog
	UF=100	**Chronic RfD = 0.0015 mg/kg/day**	
Short-Term (Dermal)	Dermal NOEL =0.56 MOE=100	Erythrocyte cholinesterase inhibition.	Dermal Absorption Rat
Intermediate-Term (Dermal)[a]	Oral NOEL=0.149 MOE=100	Erythrocyte cholinesterase inhibition.	1-Year Toxicity-Dog
Long-Term (Dermal)	Not Applicable	Not Applicable	Not Applicable
Inhalation (Any Time Period)	NOEL= 0.0012 mg/L MOE=100	Plasma and erythrocyte cholinesterase inhibition.	90-Day Inhalation Rat

[a] A 42% dermal absorption factor should be used for the intermediate-term risk assessment.

IX. REFERENCES

Short, RD; Minor, JL; Lee, CC; Chernoff, N; and Baron, RL (1980). Developmental toxicity in rats and mice. Arch. Toxicol. 43(3) pp. 177-186.

Balli, S; Yuksel, E; Ozmen, M (1996). Subchronic effects of sublethal exposure of azinphos-methyl on Swiss albino mice. Turkish Journal of Zoology 20 (Suppl) pp. 89-93.