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**Date:** **February 12, 1998**

**MEMORANDUM**

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

**FROM:** Jess Rowland
Executive Secretary,
Hazard Identification Assessment Review Committee
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
Toxicology Branch 2
Health Effects Division (7509C)

**PC Code: 101201**

On January 20, 1998, the Health Effects Division's Hazard Identification Assessment Review committee evaluated the toxicology data base of Methamidophos 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 Committee's conclusions are presented in this report.

Committee Members in Attendance

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

Other HED members also present were: William Sette of Science Analysis Branch, Sanju Diwan, Mike Ioannou, Kristina Locke and Alberto Protzel of Toxicology Branch 1, Felicia Fort, Risk Assessor of Reregistration Action Branch 2. Data was presented by Nancy McCarroll of Toxicology Branch 1.

Data Presentation: _____

Nancy McCarroll
Toxicologist

Report Preparation: _____

Jess Rowland, M.S.
Executive Secretary

## I. INTRODUCTION

On June 15, 1995, the Health Effects Division's RfD/Peer Review Committee re-assessed the Reference Dose (RfD) that was established in 1992 in light of some new data submitted by the Registrant. These data included developmental, reproductive and neurotoxicity and carcinogenicity studies. Following evaluation of these studies as well as the entire data base, the Committee concluded that the RfD should remain unchanged (Memorandum: G. Ghali, HED to W. Jacobs, RD, dated 10/6/97).

On June 29, 1995, 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. (TES Document, dated 06/22/95).

On January 20, 1998, the Health Effects Division's Hazard Identification Assessment Review committee (HIARC) met to re-assess the RfD, determine the Uncertainty Factors (UF's) and Margins of Exposure (MOE's) for dietary and non-dietary exposure risk assessments and address the potential sensitivity of infants and children from exposure to Methamidophos as required by the Food Quality Protection Act (FQPA) of 1996.

**This report supersedes the previous RfD and TES Committee reports..**

## II. HAZARD IDENTIFICATION

### A. Acute Dietary (one-day)

Studies Selected: Acute Neurotoxicity - Rat §81-8

MRID No. 43025001 and 43345801

Executive Summaries: In an acute neurotoxicity study, groups of Sprague-Dawley rats (24/sex/dose) received a single oral (gavage) administration of Methamidophos (76%) at 0, 1, 3 or 8 mg a.i/kg (actual concentrations, 0, 0.9, 3, or 9 mg a.i./kg, respectively). Functional observation battery (FOB) measurements were made (12 rats/sex/dose) at about 2 hours postdosing and on days 7 and 14. Motor and locomotor activity were determined 30 minutes after completion of the FOB on days 0, 7 and 14. Cholinesterase (ChE) activities in plasma, erythrocytes (RBC) and brain were measured at 2 hours postdosing in 6 rats/sex/dose.

For neurotoxicity, the NOEL was $<0.9$ mg/kg. The LOEL was 0.9 mg/kg (analytical value), based on slightly reduced motor/locomotor activity in males and females and clinical signs in one male consistent with neurotoxicity secondary to cholinesterase inhibition (ChEI).

For ChEI, the LOEL was 0.9 mg/kg based on inhibition of all measured activities; a NOEL was not established. Statistically significant and dose-related inhibition of serum, RBC and brain ChE was observed at all doses and in both sexes. At 1 mg/kg, ChE activity was -24% to -39% relative to control, increasing to -67% to -81% at 3 mg/kg and -82% to -92% at 8 mg/kg.

In another acute neurotoxicity study, groups of Sprague-Dawley rats (18/sex/dose) were given a single oral (gavage) dose of Methamidophos (75.6% a.i.) at doses of 0, 0.3 and 0.7 mg a.i./kg (analytical values; nominal values: 0, 0.3 and 0.6 mg a.i./kg, respectively). Neurobehavioral functions were determined in 12 rats/sex/dose at about 2 hours postdosing and on days 7 and 14. ChE activities were measured in 6 rats/sex/dose for plasma and erythrocytes at approximately 2 weeks before dosing, and in plasma, erythrocytes and brain at about 2 hours after dosing. No treatment-related effects were seen at 0.3 mg/kg. The 24% inhibition of plasma ChE activity for the females at this dose was not statistically significant and was due to an unusually high control value. This finding was, therefore, not regarded by the testing facility (and Tox. Branch/HED) as biologically relevant. At 0.7 mg/kg, there were no treatment-related effects on the neurobehavioral parameters examined, but the ChE activities were inhibited significantly ($p \leq 0.05$) in males and females (M/F) on day 0 as follows: erythrocytes (21/26%), plasma (27/25%) and brain (15/26%).

Based on the results of both studies, for neurobehavioral effects, the NOEL is 0.7 mg/kg and the LOEL is 0.9 mg/kg based on slightly reduced motor/locomotor activity in both sexes. For ChEI, the NOEL is 0.3 mg/kg/day and the LOEL is 0.7 mg/kg based on inhibition of plasma, erythrocyte and brain cholinesterase activity.

Dose and Endpoint for Risk Assessment: NOEL = 0.3 mg/kg/day based on inhibition of plasma, erythrocyte and brain cholinesterase activity at 0.7 mg/kg/day (LOEL).

Comments about Study and Endpoint: The Committee selected the dose (NOEL) based on the combined results of the two studies discussed above. ChEI occurred after a single dose (the exposure period of concern).

**This risk assessment is required.**

Acute Dietary Risk Assessment: The Committee determined that the **10 x** factor to account for enhanced sensitivity of infants and children (as required by FQPA) **should be reduce to 3 x.** Thus, for acute dietary risk assessment, **a Margin of Exposure (MOE) of 300 is required** which includes the conventional 100 and a 3 x under FQPA. **The FQPA factor is reduced** based on the following weight-of-the-evidence considerations:

(a) There is evidence of positive NTE in studies conducted with hens (See Section V, *Neurotoxicity*).

(b). Positive OPIDN has been demonstrated in studies conducted in the hen and humans (See Section V, *Neurotoxicity*).

(c) A weight-of-the-evidence evaluation of the data base indicates the need for evaluation of functional development and thus a developmental neurotoxicity study. (See Section V, *Recommendation for a Developmental Neurotoxicity Study*).

(d) Developmental toxicity studies showed no increased susceptibility in fetuses as compared to maternal animals following *in utero* exposures in rats and rabbits.

(e) A two generation reproduction toxicity study in rats showed no increased sensitivity in pups when compared to adults.

(f) The toxicology data base is complete (i.e., no data gaps for standard Subdivision F Guideline requirements).

**B. Chronic Dietary [Reference Dose (RfD)]**

The RfD established in 1992 was re-assessed by this Committee pursuant to the FQPA and is discussed below:

Study Selected: 8-Week Oral Toxicity - Rat §82-1

MRID No. 41867201

Executive Summary: Groups of Fischer 344 rats (25/dose/sex) were fed diets containing Methamidophos at dose levels of 0, 0.5, 1, 2 or 4 ppm (analytical concentrations were 0, 0.49, 0.97, 2.12 and 4.30 ppm, respectively) for 56 days. These doses were equivalent to 0, 0.03, 0.07, 0.13 and 0.24 mg/kg/day, respectively, for the males and 0, 0.06, 0.06, 0.17 and 0.28 mg/kg/day, respectively, for the females. Plasma and RBC ChE activities were determined on study days 14, 28, 42 and 51, and brain ChE activity was determined on study days 14, 35 and 56. Acetylthiocholine was used as the substrate in all of these determinations. In the case of plasma ChE activity, both acetylthiocholine and butyrylthiocholine were used as substrates. Other parameters examined were toxic signs, body weight gain and food consumption. The objective of this study was to establish a NOEL for ChEI. Treatment had no effect on survival, body weight, body weight gain or food consumption of either sex. The only systemic effect, which was observed at all dose levels and all sampling intervals, was the inhibition of ChE activities in the plasma, RBC and brain. The Data Evaluation Record, based on the decision reached by the RfD/Peer Review Committee, established the 0.03 mg/kg/day as the threshold LOEL for the inhibition of acetyl and butyryl ChE activity in plasma, and acetyl ChE activity in RBC and brain for both sexes. A NOEL was not established.

At the January 20, 1998 meeting, however, the HIARC, determined the 0.03 mg/kg/day to

be a NOEL based on the following observations. At this dose level, in males, statistically significant ChEI was not observed except in the brain ($p \leq 0.05$) at week 56 only. This finding was not considered to be biologically relevant, since the magnitude of inhibition was small (3%), appeared to be within or close to the level of detectibility of the assay and since, at previous time points at this dose level, brain ChEI of 3-5% did not reach statistical significance. Data interpretation for females fed low-dose (0.5 ppm) and mid-dose (1.0 ppm) diets was complicated by the fact that the intake of test material by these two groups was about the same (equivalent to 0.06 mg/kg/day). In low-dose group females, findings, which were of questionable biological relevance, were significant ChEI in plasma (decrease of 17% and 14%, respectively, on days 14 and 28), RBC (decreased 3% on day 42), and brain (decreased 5% on day 35). However, in the group of mid-dose females, findings were slightly pronounced. At all measurement time points, plasma cholinesterase was statistically significantly decreased (12-20%) as was brain cholinesterase (6-12%). Brain cholinesterase was also statistically significantly inhibited (7%) at all time points in mid-dose group males (equivalent to 0.07 mg/kg/day). Therefore, based primarily on brain cholinesterase decreases in both sexes at 1 ppm, the Committee selected 0.06 mg/kg/day (the equivalent dose in females) as the LOEL in this study. No effects, other than ChEI were seen in this study at dose levels up to 4 ppm (0.24 mg/kg/day and 0.28 mg/kg/day, respectively, in males and females).

Dose/Endpoint for establishing the RfD: NOEL = 0.03 mg/kg/day based primarily on brain ChEI at 0.06 mg/kg/day (LOEL).

Comments about Study and Endpoint: Although the study selected is only of a short duration (i.e., 56 days), the Committee determined that an additional UF was not necessary because both the magnitude and severity of ChE inhibition observed in the subchronic and chronic studies were comparable to that seen in this study (i.e., ChEI did not increase with time in the long-term studies).

Although data from the subchronic neurotoxicity study performed by the same laboratory in Fischer 344 rats, suggested that a slightly higher NOEL for ChEI (i.e., 0.067 mg/kg/day) might be supportable, the Committee decided that the results of the 56-day study could not be satisfactorily dismissed and should take precedence.

Uncertainty Factor (UF): 300 (see discussion below).

**Revised RfD =** $\dfrac{0.03 \text{ mg/kg/day (LOEL)}}{300 \text{ (UF)}} =$ **0.0001 mg/kg/day**

**Re-Assessment of the RfD :** At the June 15, 1992 meeting the RfD/Peer Review Committee derived the RfD by using the 0.03 mg/kg/day as the threshold LOEL and applied a UF of 30 (10 x for intra-species variation and 3 x for the lack of a NOEL). That Committee also determine that the UF to account for inter-species variation was not needed due to the existence of human data with Methamidophos and the related compound, Acephate.

At the January 20, 1998 meeting, the HIARC, after careful re-evaluation of all appropriate data, determined that the dose of 0.03 mg/kg/day is a NOEL and not a threshold LOEL; thus, there is no need to apply an additional UF (i.e., 3 x). However, the conventional UF of 10 x to account for inter-species variation should be applied because the available human data were not considered adequate to support the removal of this UF.

The HIARC determined that the **10 x** factor to account for enhanced sensitivity of infants and children (as required by FQPA) **should be reduced to 3** x. Thus, for chronic dietary risk assessment, an **UF of 300 is required** which includes 10 x for inter-species variation, 10 x for intra-species variation and 3 x for FQPA. The **FQPA factor is reduced** based on the following weight-of-the-evidence considerations:.

(a) There is evidence of positive NTE in studies conducted with hens (See Section V, *Neurotoxicity*).

(b). Positive OPIDN has been demonstrated in studies conducted in the hen and humans (See Section V, *Neurotoxicity*).

(c) A weight-of-the-evidence evaluation of the data base indicates the need for evaluation of functional development and thus a developmental neurotoxicity study. (See Section V, *Recommendation for a Developmental Neurotoxicity Study*).

(d) Developmental toxicity studies showed no increased susceptibility in fetuses as compared to maternal animals following *in utero* exposures in rats and rabbits.

(e) A two generation reproduction toxicity study in rats showed no increased sensitivity in pup when compared to adults.

(f) The toxicology data base is complete (i.e., no data gaps for standard Subdivision F Guidelines requirements).

## C. Occupational/Residential Exposure

### 1. Dermal Absorption

Dermal absorption studies are not available. Consequently, the Committee assumed a dermal absorption factor of 100% (default value) for the dermal risk assessments. This assumption is supported by the evidence of dermal absorption in the acute dermal toxicity study with rats.

In the acute dermal toxicity study, male and female rats received a single dermal application of two forms of Methamidophos (analytical and technical grades) at 0, 1, 2.5, 6.25 or 15.6 mg a.i/rat. Both forms of the test material were readily absorbed through the intact skin of both sexes of rats as evidenced, by dose-related inhibition of cholinesterase activities, cholinergic signs of toxicity and mortality (high-dose females, only). Females were more sensitive than males (MRID No. 40985208).

Dermal Absorption Factor: 100% (default value).

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

Study Selected: Developmental Toxicity - Rat §83-3a

MRID No. 43906901

Executive Summary: In a prenatal developmental toxicity study, pregnant Sprague-Dawley rats (36/group) received oral (gavage) doses of Methamidophos (76%) in distilled water at 0, 0.05, 0.14, or 5.49 mg/kg/day (analytically confirmed) during gestation days 6 through 15. Cesarean section was performed on gestation day 20. ChEI activities in plasma, RBC and brain were determined 90 minutes after administration of the last dose to the pregnant rats. For maternal toxicity, the NOEL was 0.14 mg/kg/day and the LOEL was 5.49 mg/kg/day based on decreased body weight gain and food consumption, clinical signs indicative of ChEI (tremors, muscle fasciculation, salivation), and inhibition of plasma, erythrocyte, and brain ChE. For developmental toxicity, the NOEL was 0.14 mg/kg/day and the LOEL was 5.49 mg/kg/day based on decreased placental and fetal weight, and increased skeletal variation (delayed ossification of frontal bones, sacral arches, sternebrae, and metacarpals).

Dose and Endpoint for Risk Assessment: Maternal NOEL=0.14 mg/kg/day based on cholinergic signs and ChEI (plasma, erythrocyte and brain) activity in dams at 5.49 mg/kg/day (LOEL).

Comments about the Study and Endpoint: A 21-day dermal toxicity study in rabbits is available with a systemic toxicity NOEL of 0.5 mg/kg/day and a LOEL of 5 mg/kg/day based on plasma, erythrocyte and brain ChEI. The Committee did not utilize this study because it is unacceptable for regulatory purposes due to numerous technical deficiencies. Also, based on the developmental toxicity data, there is concern for the critical endpoint (ChEI) in pregnant workers.

Since an oral dose is selected, a dermal absorption factor of 100% should be used in this risk assessment.

**This risk assessment is required.**

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

Study Selected: 8-Week Oral Toxicity - Rat §82-1

MRID No. 41867201

Executive Summary: See Chronic Dietary

Dose and Endpoint for Risk Assessment: NOEL = 0.03 mg/kg/day based primarily on brain ChEI at 0.06 mg/kg/day (LOEL).

Comments about Study and Endpoint: See Short-Term Dermal. Since an oral dose is selected, a dermal absorption factor of 100% should be used in this risk assessment.

**This risk assessment is required.**

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

Study Selected: 8-Week Oral Toxicity - Rat §82-1

MRID No. 41867201

Executive Summary: See Chronic Dietary

Dose/Endpoint for establishing the RfD: NOEL = 0.03 mg/kg/day based primarily on brain ChEI at 0.06 mg/kg/day (LOEL).

Comments about Study and Endpoint: See Short-Term Dermal. This dose/endpoint was also used in deriving the RfD. Since an oral dose is selected, a dermal absorption factor of 100% should be used in this risk assessment.

**This risk assessment is required.**

**5. Inhalation Exposure (Any-Time period)**

Study Selected: Subchronic Inhalation Toxicity - Rat §82-4

MRID No. 41402401

Executive Summary Groups of Wistar rats (10/sex/concentration) were exposed (head/nose only) to aerosol concentrations of Methamidophos (73.4%) at 0, 1.1, 5.4 and 23.1 mg/m$^3$ (0, 0.001, 0.005 and 0.023 mg/L, respectively) 6 hours/day, 5 days/week for 3 months. There were also two satellite groups (10 rats/sex/group) which served as recovery groups. No treatment-related effects were observed in the low-dose group. Effects in the mid-dose males and females were limited to RBC (7-28%; P<0.05 or 0.01) and plasma (44-63%; P<0.05 or 0.01) ChEI throughout the treatment period and brain ChEI (25-29%; P<0.01) at the end of the study. There was no substantive difference in the magnitude of the response on plasma or RBC ChEI from weeks 1-13. The following effects were observed in the high-dose male and female rats when compared with the vehicle control rats: (1) slight to moderate muscle tremors and aggressive behavior; (2) decreased body weight gain (53%); (3) decreased food consumption (5-28%); (4) increased plasma lactate dehydrogenase (63%) and glutamate oxaloacetate transaminase (32%) activities in males only; (5) decreased plasma protein (8%), cholesterol (16-19%) and glucose concentrations (10-11%); (6) inhibition of ChE activities in erythrocytes (15-44%; P<0.05 or 0.01) and plasma (53-93%; P<0.01) throughout the treatment period and brain (45-47%) at study termination; there was no substantive difference in the magnitude of the response on plasma or erythrocyte ChE inhibition from weeks 1-13 and (7) decreased spleen weight, both absolute (15-25%; P<0.01) and relative (organ/body weight ratio, 11%; P<0.01). When treatment was discontinued, ChE activities in the RBC and plasma (not determined in the brain) returned to the pretreatment values. For systemic toxicity, the NOEL was 5.4 mg/m$^3$ (0.005 mg/L) and the LOEL was 23.1 mg/m$^3$ (0.0231 mg/L) based on clinical signs, decreased body weight gain and feed consumption, altered clinical chemistry parameters, and decreased spleen weights. For ChEI, the NOEL was 1.1 mg/m$^3$ (0.001 mg/L) and the LOEL was 5.4 mg/m$^3$ (0.005 mg/L) based on inhibition of ChE activities in RBC, plasma and brain.

Dose/Endpoint for establishing the RfD: NOEL = 0.001 mg/L based on plasma, RBC and brain ChEI at 0.005 mg/L (LOEL).
Comments about Study and Endpoint: Since this is the only study available, the dose selected from this study should used for inhalation risk assessments for any time period (i.e., Short-, Intermediate, and Long-Term).

**This risk assessment is required.**

**D. Margin of Exposure for Occupational/Residential Exposures:**

For Short-, Intermediate, and Long-Term dermal and inhalation exposure risk assessments, **a MOE of 300 is required** and includes the conventional 100 x and the FQPA 3 x. The rationale for reducing the FQPA factor to 3 x and thus the requirement for a MOE of 300 is provided in Section I.*Acute Dietary*.

**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.

$$\text{Total MOE: } \frac{1}{\frac{1}{MOE_{Oral}} + \frac{1}{MOE_{Dermal}} + \frac{1}{MOE_{Inhalation}}}$$

## III. CLASSIFICATION OF CARCINOGENIC POTENTIAL

At the June 1995, meeting the RfD/Peer Review Committee classified Methamidophos 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 Nos. 00145579, 00147937 & 43248101) and in male and female Fischer 344 rats (MRID Nos. 00148952 & 43248102). The HIARC re-affirmed the previous classification.

## IV. MUTAGENICITY

1. Gene Mutations

In a *Salmonella typhimurium* reverse gene mutation assay, independent trials were negative up to the highest concentration tested (10,000 µg/plate) with or without metabolic (S9) activation (MRID No. 00098457).

A Chinese hamster ovary (CHO) cell HGPRT forward gene mutation assay gave negative results up to the highest nonactivated dose tested (5000 µg/mL); however, dose-related increases in the mutation frequency (MF) were seen at 4000 & 5000 µg/mL +S9 (MRID No. 42854701). The evidence is considered weak but consistent with the findings of a previously conducted CHO/HGPRT assay (MRID No. 42013701). This earlier study was classified as Unacceptable because definitive conclusions could not be reached regarding the slight increases in the MF at high S9-activated doses.

2. Chromosome Aberrations

An *in vitro* chromosome aberrations assay in CHO cells gave positive results; reproducible and significant increases in structural chromosome aberrations were obtained at 4200 and 5140 μg/mL without S9 but only after 20 hours of continuous exposure. In the presence of S9 activation, there was no indication of a clastogenic response; however, nondose-related increases in polyploidy were noted at 3150, 4200 & 5250 μg/mL (MRID No. 41461401).

In an *in vivo* bone marrow cytogenetic assay there was no evidence of mutagenicity in CD-1 male and female mice given a single oral (gavage) administration at doses ranging from 0.6-12 mg/kg active ingredient (a.i.). Toxic signs, consistent with cholinesterase poisoning, were observed at 6, 9 and 12 mg/kg a.i. Lower levels (≤2 mg/kg a.i.) were not overtly toxic and there was, no evidence of a cytotoxic effect on the target cell at any dose (MRID No. 41234306).

3. Other Mutagenic Mechanisms

Negative results were obtained in an *in vitro* unscheduled DNA synthesis in primary rat hepatocytes treated up to cytotoxic concentrations (>1 μL/mL) (MRID No. 41234305).

4. Conclusions:

The available studies indicate that Methamidophos is not mutagenic in bacteria but does induce gene mutations in cultured mammalian cells at high S9-activated levels. Similarly, there was evidence of clastogenicity at high nonactivated concentrations and polyploidy at high S9-activated doses. In contrast, Methamidophos was negative for chromosome aberrations *in vivo* and did not induce UDS *in vitro*. The data suggest, therefore, that the marginal genotoxicity activity seen with the test substance is not expressed *in vivo*. The lack of an oncogenic effect in the rat or mouse long-term feeding studies and the absence of significant reproductive or developmental toxicity that could be associated with a mutagenic mode of action (i.e., germ cell damage, reduced numbers of pregnancies, decreased total implants, increased resorptions) support this conclusion. Based on these considerations, the Committee concluded that there is no concern for mutagenicity.

## V. FQPA CONSIDERATIONS

1. Neurotoxicity Data

(i) Hen

In an acute oral delayed neurotoxicity study White Leghorn hens were exposed to Methamidophos technical (74% a.i.). In the preliminary study (acute oral lethality), groups of six hens received a single oral doses of 10, 15, 22.5, 33.75, 50.63 or 75.94 mg a.i./kg and were observed for 42 days. In the main study, hens were dosed orally with 30 (10 hens) or 50.63 mg a.i./kg (12 hens) and atropine sulfate (50 mg/kg) on day 0. Survivors were redosed on day 21. As a positive control, 10 hens were administered 500 mg/kg triothrocresophosphate (TOCP) and 16 hens served as untreated controls. Parameters examined included: daily observations, body weight and feed consumption every third day, gross necropsy, and histological evaluation of the perfused spinal cord and peripheral nerves. Neither forced motor activity nor neurotoxic esterase (NTE) were assessed (MRID 00041317).

In the oral lethality study, deaths (>2 hours-6 days) and other signs of toxicity were observed at ≥22.5 mg/kg. Acute signs of poisoning included: muscular weakness, unsteadiness (leg weakness), diarrhea, excessive salivation, anorexia, lateral and sternal recumbency, dyspnea, and cyanotic combs and wattles shortly before death. The higher the dose, the sooner the onset of toxic signs and death. Death was caused by respiratory paralysis. No signs of toxicity were observed at 10 or 15 mg/kg. Based on these findings, the oral $LD_{50}$ in hens = 29.75 mg/kg. In the main study, no signs of delayed toxicity or histopathological lesions typical of delayed neurotoxicity were observed at 30 or 50.63 mg/kg. Two of 10 hens died at 30 mg/kg and 4/12 hens died at 50.63 mg/kg. Signs of delayed toxicity and histopathological lesions typical of delayed neurotoxicity were observed in 7/10 positive control hens dosed with 500 mg/kg TOCP (MRID 00041317).

In a subchronic delayed neurotoxicity study, Methamidophos (76% a.i) was administered to 16 White Leghorn hens/dose by oral gavage 5 days/week for 3 months at dose levels of 0, 0.3, 1 or 3 mg/kg/day. The highest dose tested (3 mg/kg/day) was based on the results of a preliminary study. Parameters examined included: (1) twice daily clinical signs and mortality, twice weekly forced motor activity and weekly body weights--all hens, (2) plasma butyrylcholinesterase (BuChE) activity at pretest and during weeks 4, 8 and 12, (3) necropsy and histology of perfused brain, spinal cord and peripheral nerves--10 hens/group (4) neuropathy target esterase (neurotoxic esterase, NTE) in the brains and spinal cords of 6 hens/group; these hens were not examined grossly or microscopically (MRID 40985202).

Treatment-related findings observed in the 3 mg/kg/day group included somnolence,

emaciation, weight loss (22%), and significant inhibition of BuChE activity in plasma at weeks 4, 8 and 12 (average of all means = 48%) and NTE activity in brain (17%) and spinal cord (42%). At 1 mg/kg/day, no clinical signs or weight loss were noted. A nonsignificant inhibition of plasma BuChE (23%) was seen at week 4 and significant inhibition was reported for this dose group at week 8 (27%); the average of all means was 22% but not significant. Also at 1 mg/kg/day, NTE activity was significantly inhibited in the spinal cord (22%) but not the brain (2%). No effects on clinical signs, body weight, plasma BuChE or NTE occurred at 0.3 mg/kg/day (MRID 40985202. Overall, the data indicate that BuChE inhibition was dose related in the mid- and high-dose groups; the peak response appeared to occur at week 8. Similarly, NTE inhibition was dose related at 1 and 3 mg/kg. However, ataxia, abnormal motor activity or histological changes in brain, spinal cord and peripheral nerves, generally regarded as indicators of delayed neurotoxicity, were not observed in any hen on the study. Based on the negative results of the forced motor activity tests and microscopic examinations of brain, spinal cord and peripheral nerves, Methamidophos did not induce delayed neurotoxicity in hens. The NOEL was 0.3 mg/kg/day and the LOEL was 1 mg/kg based on inhibition of plasma BuChE and spinal cord NTE activity (MRID 40985202).

(ii) Rats

In an acute neurotoxicity screening study, Methamidophos technical (≈76%) was administered in a single gavage dose to 24 male and 24 female Sprague-Dawley rats at 0, 1, 3 or 8 mg a.i./kg. Actual concentrations, based on analytical determinations, were 0.9, 3, or 9 mg a.i./kg, respectively. Parameters examined included: (1) daily observations for changes in clinical condition, (2) body weights -- weekly as part of the functional observational battery (FOB), (3) FOB for 12 rats/sex/dose at about 2 hours postdosing and on days 7 and 14, (4) motor and locomotor activity 30 minutes after completion of the FOB on days 0, 7 and 14, (5) cholinesterase (ChE) activities in plasma, erythrocytes (RBC) and brain at 2 hours postdosing in 6 rats/sex/dose, (6) standard hematology and clinical chemistry on day 1 in 6 rats/sex/dose, and (7) brain weights for 6 rats/group/dose (FOB groups) and histological examinations on perfused brain (6 levels - olfactory region, forebrain, midbrain, pons, medulla oblongata, and cerebellum), spinal cord and peripheral nerves (MRID 43025001).

At 1 mg/kg, males had slightly decreased motor/locomotion activities (-23 to -25% less than controls but not statistically significant) and one male had clinical signs (increased sitting/lying; urine, oral and nasal staining). Females at this dose showed slightly reduced motor activity (-26%) during the first interval. At 3 mg/kg, markedly decreased motor/locomotor activity (-84 to -96%), repetitive chewing, uncoordinated gait, muscle fasciculation, impaired righting reflex, decreased forelimb grip strength (80% of control), decreased activity and rearing, increased ease of removal from cage and decreased body temperature were observed. Males at 3 mg/kg also had ataxia, reduced approach or touch response and increased SGOT activity (143% of control), and females had increased lateral recumbency and tremors and decreased triglycerides (56% of control). At 8 mg/kg, salivation, flattened posture, reduced clicking sound or tail pinch responses and increased SGPT (170-181% of control) were observed. Males

also had tremors and increased serum cholesterol (130% of control) and females had decreased hindlimb grip strength (71% of control), reduced approach and touch response and increased SGOT (67% of control). Most clinical signs were observed only on the day of dosing and were completely resolved by study day 5. The peak effect on FOB and motor and locomotor activities occurred on day 0. No treatment related gross or histopathological effects were seen; brain weights were unaffected by treatment (MRID 43025001).

For neurotoxicity, the NOEL was <0.9 mg/kg and the LOEL was 0.9 mg/kg (analytical value), based on slightly reduced motor/locomotor activity in males and females and clinical signs in one male consistent with neurotoxicity secondary to cholinesterase inhibition (MRID 43025001).

For ChEI, the LOEL was 0.9 mg/kg (analytical value), based on inhibition of all measured activities; a NOEL was not established. Statistically significant and dose-related inhibition of serum, RBC and brain ChE was observed at all doses and in both sexes. At 1 mg/kg, ChE activity was -24% to -39% less than control, increasing to -67% to -81% at 3 mg/kg and -82% to -92% at 8 mg/kg (MRID 43025001).

In another acute neurotoxicity screening study, Methamidophos (75.6% a.i.) was administered in a single gavage dose to 18 male and 18 female Sprague-Dawley rats at 0, 0.3 and 0.7 mg a.i./kg (analytical values; nominal values: 0, 0.3 and 0.6 mg a.i./kg, respectively). Twelve rats/sex/dose were assessed for neurobehavioral functions at about 2 hours postdosing and on days 7 and 14. Six rats/sex/dose were used for the determination of cholinesterase (ChE) activities in plasma and erythrocytes at ≈ 2 weeks before dosing, and in plasma, erythrocytes and brain at about 2 hours after dosing; ChE activities were not measured during the remaining 13 days. Other parameters examined were clinical observations and body weights. Gross necropsy and histopathology were not performed because nothing remarkable was observed in the previous study discussed above (MRID 43345801).

No treatment-related effects were seen at 0.3 mg/kg. The 24% inhibition of plasma ChE activity for the females in this group was not statistically significant and was due to an unusually high control value. This finding was, therefore, not regarded by the testing facility (and Tox. Branch/HED) as biologically relevant (MRID 43345801).

At 0.7 mg/kg, no effect were seen in the neurobehavioral parameters examined, but the ChE activities were inhibited significantly ($p \leq 0.05$) in males and females (M/F) on day 0 as follows: erythrocytes (21/26%), plasma (27/25%) and brain (15/26%) (MRID 43345801).

Based on the results of both studies, for neurobehavioral effects, the NOEL was 0.7 mg/kg and the LOEL was 0.9 mg/kg. For ChEI, the NOEL was 0.3 mg/kg/day and the LOEL was 0.7 mg/kg, respectively (MRID 43345801).

In a subchronic neurotoxicity study, groups of Fischer 344 rats (18/sex/dose) were fed diets containing Methamidophos (75.6-75.8%) at dose levels of 0, 1, 12 or 60 ppm a.i. (analytical concentrations were 0, 1, 12 or 59 ppm a.i., respectively) for 13 weeks. Dose selection was based on the results of subchronic and chronic feeding studies. The doses used in this subchronic neurotoxicity screening study were equivalent to 0, 0.067, 0.787 or 4.25 mg/kg/day, respectively (males) and 0, 0.074, 0.899 or 4.94 mg/kg/day, respectively (females). Parameters examined included: (1) daily observations for changes in clinical condition, (2) body weights -- weekly and at termination, (3) weekly feed consumption, (4) functional observational battery (FOB) for 12 rats/sex/dose at 1 week prior to dosing and on weeks 4, 8 and 13, (5) motor and locomotor activity as above for the FOB, (6) ophthalmological examinations before treatment and during week 12 --all rats, (7) cholinesterase (ChE) activities in plasma and erythrocytes (RBCs) prior to treatment and during weeks 4 and 13 and brain at study termination in 6 rats/sex/dose (FOB groups), (8) gross necropsy and brain weights for 6 rats/sex/dose (FOB groups), and (9) histological examinations on perfused brain (6 levels - olfactory region, forebrain, midbrain, pons, medulla oblongata, and cerebellum), spinal cord, both eyes with optic nerves, peripheral nerves, gasserian ganglion, gastrocnemius muscle and tail. (MRID 43197901),

Treatment-related clinical signs in males and females of the high-dose group included: muscle fasciculation, increased reactivity, perianal and urine staining, and red and clear lacrimation; tremors were also noted in the high-dose males. Reductions in motor and locomotion activities (26-57% of controls -statistically significant in males at all test intervals and females during week 4) and decreased forelimb grip strength (14-31% of control - statistically significant in males at all test intervals and females during weeks 8 and 13) were also reported. There was no evidence of cumulative toxicity beyond week 8. Reduced activity (sluggish arousal during open field observations) was only seen in the high-dose females. Decreased body weight gain was also recorded for the high-dose males (11%) and females (17%). Mid-dose females had an increased incidence of urine stains throughout most of the study. Other treatment-related effects in the mid-dose group were: reduced motor and locomotor activities (17-32% of control--both sexes) and decreased body weight gain in the females (10%). No treatment-related effects on clinical signs, motor and locomotor activities, FOB or body weight were observed in the low-dose group. Similarly, treatment with Methamidophos had no adverse effects on the incidence of gross or microscopic changes or brain weights For neurotoxicity, the NOEL was 1 ppm (0.067 mg/kg/day for males and 0.074 mg/kg/day for females) and the LOEL was 12 ppm (0.787 mg/kg/day for males and 0.889 mg/kg/day for females) (MRID 43197901).

The ChE data indicate that inhibition of plasma and RBC ChE was statistically significant and dose related in both sexes at the mid- and high-dose and at both sampling times (weeks 4 and 13). Significant inhibition of plasma ChE was also observed in low-dose females at week 4. Brain ChE inhibition was dose related and significant in males of all treatment groups and females of the mid- and high-dose groups. At the highest dose tested (59 ppm, analytical value), brain ChE was inhibited 84-86%, plasma ChE inhibition

ranged from 74-91% and RBC ChE ranged from 79-98%. In the mid-dose group, brain ChE was inhibited 58-60%, plasma ChE was suppressed 41-64% and RBC ChE activity was inhibited 70-77%. At the lowest dose tested (1 ppm, analytical value), brain ChE was inhibited by 6%, plasma ChE by 6-26% and RBC ChE by 1-9%. The NOELs and LOELs for inhibition of ChE (both sexes) were: RBC: NOEL = 1 ppm (males, 0.067 mg/kg/day and females, 0.074 mg/kg/day) LOEL = 12 ppm (males, 0.787 mg/kg/day and females, 0.899 mg/kg/day). Plasma and brain: NOEL = <1 ppm (males, <0.067 mg/kg/day and females, <0.074 mg/kg/day, lowest dose tested) LOEL = 1 ppm (MRID 43197901).,

(iii) Special Studies with Methamidophos: Racemate and Enantiomers

Methamidophos is a racemic mixture of two stereoisomers: dextrorotary D (+) and levorotary L (-). This racemic mixture, Methamidophos (+ -), can be separated into the individual isomers (enantiomers): Methamidophos (+) and Methamidophos (-). The three compounds were the subject of the special studies summarized below:

In an acute oral toxicity study, the LD50s for male Wistar rats were: Methamidophos (+/-): 16 mg/kg; (+) isomer: 14 mg/kg; and (-) isomer: 16 mg/kg. Toxic signs characteristic of ChEI were seen with all three test materials (MRID No. 41685802).

In an acute oral toxicity study, the LD s FOR hens were: Methamidophos (+/-): 25 mg/kg; (+) isomer: 43 mg/kg; and (-) isomer: 82 mg/kg. Toxic signs characteristic of ChEI were seen with all three test materials (MRID No. 41685803).

In a NTE study in hens, inhibition of NTE activity in the brain was: 66% at twice the $LD_{50}$ (50 mg/kg) for Methamidophos (+/-) with 89% of the inhibited NTE being reactivated in an unmodified (unaged) form. For the Methamidophos (+) isomer, NTE was inhibited 98% at ten times the $LD_{50}$ (400 mg/kg) with 86% of the inhibited NTE being reactivated in an unaged form. For the Methamidophos (-) isomer, NTE was inhibited by 58-84% at five times the $LD_{50}$ (400 mg/kg) with 27% of the inhibited NTE being reactivated in an unaged form. Approximately 73% of the inhibited NTE was modified (aged). Based on the marked percentage of aged NTE, the (-) isomer could be considered a possible trigger for organophosphorus esterase-induced delayed polyneuropathy (OPIDP) (MRID No. 41685804).

In an OPIDP study in hens, Methamidophos (+/-) was positive for OPIDP at 400 mg/kg (16x $LD_{50}$); the Methamidophos (+) isomer was positive for OPIDP at 400 mg/kg (9x $LD_{50}$); and the Methamidophos (-) isomer was negative for OPIDP at 400 mg/kg. However, only two hen were available for the assessment of OPIDP with the (-) isomer (MRID No. 41685805).

(iv) Information from the Open Literature

Based on an earlier review of data from 1961-1980 presented in the open literature, ingestion of high doses of Methamidophos (usually as a result of suicide attempts but occasionally by accident) can cause delayed neurotoxicity (polyneuropathy) in humans. Similarly, adult hens can develop polyneuropathy but only after ingesting Methamidophos levels equivalent to 12-16 times the $LD_{50}$. Based on these considerations, it was assessed that Methamidophos does have delayed neurotoxic potential but only at excessive, life threatening concentrations (MRID No. 41685801).

A survey of the more recent literature supports the above statements and suggests that humans may be sensitive to peripheral neuropathy following exposure to high doses of Methamidophos (through accidental occupational poisonings, suicide attempts, or ingestion of contaminated vegetables). Effects cited include: elevated vibrotactile threshold among agricultural workers (McConnell, et al.,1994) and delayed polyneuropathy following acute Methamidophos toxicosis (Zheng, 1990).

2. Determination of Susceptibility

There was no indication of increased susceptibility of the offspring of rats or rabbits to pre- and or postnatal exposure to Methamidophos. In all studies examined, maternal or parental NOELs were less than or equivalent to offspring NOELs.

(i) Developmental Toxicity:

In a prenatal developmental toxicity study, pregnant Sprague-Dawley rats (24-27/group) received oral (gavage) administration of Methamidophos (70.5%) in distilled water at dose levels of 0, 0.3, 1.0, or 3.0 mg/kg/day during gestation days 6 through 15. Cesarean section was performed on gestation day 21. For maternal toxicity, the NOEL was 1.0 mg/kg/day and the LOEL was 3.0 mg/kg/day based on decreased body weight gain and food consumption and clinical signs indicative of cholinesterase inhibition (fasciculation, hyperactivity, salivation, lacrimation). For developmental toxicity, the NOEL was 1.0 mg/kg/day and the LOEL was 3 mg/kg/day based on decreased fetal weight (MRID 00148454).

The other prenatal developmental toxicity study in Sprague-Dawley rats is discussed in Section II. Short-Term Dermal (MRID No. 43906901).

In a prenatal developmental toxicity study conducted in Himalayan rabbits, does (16/dose) received oral (gavage) administration of Methamidophos (62%) in aqueous 0.5% cremophor at doses of 0.1, 0.5, or 2.5 mg/kg/day during gestation days 6 through 18. A NOEL for maternal toxicity was not established (<0.1 mg/kg/day), based upon decreased body weight gain during gestation at the lowest dose tested (maternal LOEL = 0.1 mg/kg/day). No developmental toxicity was observed (developmental NOEL $\geq$2.5 mg/kg/day) (MRID 00041315).

In another prenatal developmental toxicity study in New Zealand white rabbits, does (23/dose) received oral (gavage) administration of Methamidophos (76%) in deionized water at nominal doses of 0.1, 0.5, or 2.5 mg/kg/day (analytically confirmed as 0.2, 0.65, or 2.47 mg/kg/day) on gestation days 6-18. For maternal toxicity, the NOEL was 0.20 mg/kg/day and the LOEL was 0.65 mg/kg/day based upon decreased body weight gain and food consumption during gestation. At the HDT (2.5 mg/kg/day, hyperactivity and body weight loss were also observed. No developmental toxicity was observed. For developmental toxicity, the NOEL was ≥2.5 mg/kg/day; a LOEL was not established (MRID 44040601).

(ii) Reproductive Toxicity:

In a two-generation reproduction study, Sprague Dawley rats received diets containing Methamidophos (70.5%) at concentrations of 0, 3, 10, or 33 ppm (approximately equivalent to 0, 0.15, 0.5 or 1.65 mg/kg/day, respectively). For parental systemic toxicity, the NOEL was 10 ppm (0.5 mg/kg/day) and the LOEL was 33 ppm (1.65 mg/kg/day), based on decreased body weight of males and females during premating and of females during lactation. For reproductive toxicity, the NOEL was also 10 ppm (0.5 mg/kg/day) and the LOEL was 33 ppm (1.65 mg/kg/day) based upon the decreased number of females giving birth. For offspring toxicity, the NOEL was 10 ppm (0.5 mg/kg/day) and the LOEL was 33 ppm (1.65 mg/kg/day) based on decreased pup viability and body weight during lactation (MRID 00148455).

3. Recommendation for a Developmental Neurotoxicity Study

Based on a weight-of-the-evidence evaluation, the Committee determined that an assessment of functional development is necessary to fully evaluate the effects of Methamidophos exposure on perinatal animals; therefore, a developmental neurotoxicity study in rats with Methamidophos is **required**. The following information was considered in support of this decision:

Evidence that support requiring a developmental neurotoxicity study:

- Methamidophos is a neurotoxic chemical and the concern for SAR chemical class; Methamidophos is an organophosphorus chemical.
- Administration to various species (rat, dog, mouse, human) results in ChE inhibition. Frank neurobehavioral observations generally occur at a level that is only slightly higher than the dose at which ChEI is first observed.
- Methamidophos is acutely lethal at relatively low doses, with an oral LD50 value of approximately 16 mg/kg in the rat.

- In studies from the open literature, Methamidophos ingestion has been shown to result in delayed peripheral neuropathy in humans and polyneuropathy in hens, albeit at extremely high dose levels (in excess of the hen $LD_{50}$). It was recognized by the Committee, however, that the dose levels causing the human delayed neuropathy are not well characterized.

- NTE evaluations were positive in the subchronic neurotoxicity study in hens and also in a hen study from the open literature.

Evidence that do not support asking for a developmental neurotoxicity study:

- No evidence of abnormalities in the development of the fetal nervous system were observed in the prenatal developmental toxicity studies at maternally toxic oral doses up to 5.49 mg/kg/day (rats) or 2.5 mg/kg/day (rabbits).

- There was no evidence of behavioral abnormalities or other findings suggestive of neurotoxicity in the offspring of the 2-generation reproduction study in rats.

- Neither brain weights nor histopathology (perfused or nonperfused) of the central or peripheral nervous system of several species was affected in the subchronic and chronic studies. Similar results were obtained in the neurotoxicity studies in rats.

- Delayed peripheral neuropathy in humans was shown to occur only following exposure to life threatening doses. Exposure of agricultural workers at lower levels did not result in OPIDN. It is noted that some Committee members expressed concerns that the evidence of neuropathy in hens and humans at high doses is not predictive of potential effects on functional development and that the developmental neurotoxicity study in rats would not provide an adequate model to assess effects on perinatal exposure.

NOTE: The recommendation for a developmental neurotoxicity study this is a reversal of the decision made at the RfD Peer Review Committee meeting of June 15, 1995, at which time a developmental neurotoxicity study was not recommended.

4. Determination of Uncertainty Factor:

The Committee determined that the **10 x** factor to account for enhanced sensitivity of infants and children (as required by FQPA) **should be reduce to 3 x** and is based on the following weight-of-the-evidence considerations:

(a) There is evidence of positive NTE in studies conducted with hens (See Section V, *Neurotoxicity*).

(b). Positive OPIDN has been demonstrated in studies conducted in the hen and humans (See Section V, *Neurotoxicity*).

(c) A weight-of-the-evidence evaluation of the data base indicates the need for evaluation of functional development and thus a developmental neurotoxicity study. (See Section V, *Recommendation for a Developmental Neurotoxicity Study*).

(d) Developmental toxicity studies showed no increased susceptibility in fetuses as compared to maternal animals following *in utero* exposures in rats and rabbits.

(e) A two generation reproduction toxicity study in rats showed no increased sensitivity in pup when compared to adults.

(f) The toxicology data base is complete (i.e., no data gaps for standard Subdivision F Guideline requirements).

## VI. DATA GAPS

None for standard Subdivision F Guideline requirements; however, the Committee has determined that a developmental neurotoxicity study in rats is required.

## VII. SUMMARY OF TOXICOLOGY ENDPOINT SELECTION

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

| EXPOSURE SCENARIO | DOSE (mg/kg/day) | ENDPOINT | STUDY | MOE REQUIRED |
|---|---|---|---|---|
| Acute Dietary | NOEL =0.3 | Brain cholinesterase inhibition | Acute Neurotoxicity- Rat | 300 |
| Chronic Dietary | NOEL=0.03 | Brain cholinesterase inhibition. | 8-Week Toxicity- Rat | 300 |
| | **Revised RfD = 0.0001 mg/kg/day** | | | |
| Short-Term (Dermal) | Developmental NOEL =0.25 | Plasma, erythrocyte and brain cholinesterase inhibition. | Developmental Toxicity - Rat | 300 |
| Intermediate-Term (Dermal) | Oral NOEL=0.03 | Brain cholinesterase inhibition. | 8-Week Toxicity-Rat | 300 |
| Long-Term (Dermal) | Oral LOEL=0.03 | Brain cholinesterase inhibition. | 8-Week Toxicity-Rat | 300 |
| Inhalation (Any Time Period) | NOEL= 0.001 mg/L | Plasma, erythrocyte and brain cholinesterase inhibition. | 90-Day Inhalation Rat | 300 |

## VIII. REFERENCES

McConnell, R., Keifer, M., Rosentock, L. (1994). Elevated quantitative vibrotactile threshold among workers previously poisoned with methamidophos and other organophosphate pesticides. Am. J. Ind. Med. 25(3) p 325-334.

Zheng, R.Y. (1990). Clinical features of delayed polyneuropathy induced by acute methamidophos toxicosis in 74 cases. Chung-Hua-Nei-Ko-Tsa-Chin 29(2) p 79-82.