Appendix I HED Effects

1. Phorate Hazard Assessment

a. Acute Toxicity

There are few new acute toxicity studies available for phorate. Essentially all the acute toxicity studies were previously reviewed and published in the Registration Standard for phorate (December, 1988). The acute toxicity data base is adequate for phorate. Table 1 summarizes acute toxicity values and categories for phorate.

Table 1. Acute Toxicity Values for Technical Phorate1

Study	Results	Category
Oral LD50 - Rat	3.7 mg/kg (M), 1.4 mg/kg (F)	I
Dermal LD50 - Rat	9.3 mg/kg (M), 3.9 mg/kg (F)	I
Inhalation LC50- Rat	0.06 mg/L (M), 0.011 mg/L (F)	I
Eye Irritation	Waived2	N/A
Dermal Irritation	Waived2	N/A
Dermal Sensitization	Waived2	N/A

1 Data are excerpted from the Pesticide Registration Standard for Phorate (Dec. 1988)(p. 8-9). 2 High acute toxicity prohibits administration of appropriate dose levels.

Technical phorate is highly toxic on an acute oral, dermal, and inhalation basis. The oral LD50 values for phorate with rats were 3.7 and 1.4 mg/kg in males and females, respectively (Toxicity Category I). All of the animals that died in this study showed typical clinical signs of cholinergic toxicity such as salivation, lacrimation, exophthalmos, muscle fasciculation andexcessive urination and defecation (US EPA, 1988; Newell and Dilley, 1978; MRID# 00126343; satisfies Guideline 870.1100).

The dermal LD50 values for phorate with rats were 9.3 and 3.9 mg/kg in males and females, respectively (Toxicity Category I). The cholinergic signs noted for the acute oral study were also observed in the acute dermal study (US EPA, 1988; Newell and Dilley, 1978; MRID# 00126343; satisfies Guideline 870.1200). In addition, a dermal LD50 of 415.6 mg/kg in guinea pigs with typical cholinergic signs noted at higher doses was also reported (Shaffer, 1960; Baron, 1968; MRID# 00139479).

The acute inhalation LC50s for rats were 0.06 and 0.011 mg/L for males and females, respectively (Toxicity Category I), based on a one-hour exposure to analytical grade phorate aerosol. Typical cholinergic signs were observed in intoxicated animals (US EPA, 1988; Newell and Dilley, 1978; MRID# 00126343; satisfies Guideline 870.1300).

There were no acceptable data available on the primary eye or dermal irritation properties of phorate. However, these tests were waived since the high acute toxicity of phorate prohibits the administration of appropriate dosage levels. Likewise, no data are available on the primary dermal sensitization properties of phorate. This study was waived because of the high acute toxicity of phorate (US EPA, 1988).

b. Subchronic Toxicity

There were data available from a 90-day feeding study in rats and a 105-day feeding study in dogs (MRID# 00092873). These studies were conducted in 1956 and were classified as supplementary since the protocols did not adhere to the current guidelines. However, because the toxicity endpoint (cholinesterase inhibition) was satisfactorily identified, and because sufficient data from chronic toxicity studies in rodents and non-rodents were available, additional data from subchronic toxicity studies are not required. Executive summaries of theses two studies follow.

In a 90-day feeding study in rats (Tusing, 1956; MRID# 0092873), phorate was administered in the diet at dosage levels of 0, 0.22, 0.66, 2.0, 6.0, 12.0 or 18.0 ppm (equivalent to 0, 0.011, 0.033, 0.1, 0.3, 0.6, and 0.9 mg/kg/day, respectively) for 90 days. Phorate at 12 and 18 ppm induced mortality as well as reduced body weight gains and food consumption (both sexes). RBC ChE activity was inhibited in females at 2.0 ppm while plasma, RBC and brain ChE activities were inhibited in both sexes at the 6.0 ppm level. The NOAEL was 0.66 ppm (0.033 mg/kg/day) and the LOAEL was 2 ppm (0.1 mg/kg/day) based on cholinesterase inhibition. The study was classified as supplementary because the histopathology was performed on only 3 (not 10) rats/sex.

In a 105-day feeding study in dogs (Tusing, 1956; MRID# 0092873), technical phorate was administered in capsules to dogs at dosages of 0, 0.01, 0.05, 0.25, 1.25 or 2.5 mg/kg/day, 6 days/week for 13-15 weeks. Each group had 3 dogs (2 males and 1 female) with the exception of the 2.5 mg/kg group, which had 2 males only. The plasma ChE activity was inhibited at a dose of 0.05 mg/kg/day or above (combined sexes). The RBC ChE was inhibited at a dose of 0.25 mg/kg/day or above (combined sexes). All dogs at the 1.25 and 2.5 mg/kg/day levels showed typical cholinergic signs and subsequently died. The NOAEL was 0.01 mg/kg/day and the LOAEL was 0.05 mg/kg/day based on the reduction of plasma ChE activity. This study was classified as supplementary because only three dogs (2 males and 1 female) per group were used instead of 4 dogs of each sex per group (8 dogs total).

No data are available from 21-day or 90-day dermal toxicity studies with phorate. These study requirements were waived since the highly toxic nature of phorate prohibits the administration of dosages that could induce adverse effects other than inhibition of cholinesterase activity (US EPA, 1988).

c. Chronic Toxicity and Carcinogenicity

In a combined two-year chronic toxicity/carcinogenicity study in rats (50/sex/group), phorate was administered in the diet (50/sex/group) at dosage levels of 0, 1, 3, or 6 ppm (equivalent to 0, 0.05, 0.15, and 0.3 mg/kg/day, respectively) for 24 months. A NOAEL for plasma ChE inhibition in males was not established since the LOAEL was 0.05 mg/kg/day, the lowest dose tested (LDT). The NOAEL for plasma ChE inhibition in females was 0.05 mg/kg/day while the LOAEL was 0.15 mg/kg/day. The NOAEL for RBC ChE inhibition was 0.3 mg/kg/day (highest dose tested (HDT)) in males and 0.15 mg/kg/day in females while the LOAEL for females was 0.3 mg/kg/day. The NOAEL for brain ChE inhibition was 0.15 mg/kg/day in males and 0.05 mg/kg/day in females while the LOAELs were 0.3 and 0.15 mg/kg/day for males and females, respectively. The high dose level tested was considered adequate for carcinogenicity testing.

Phorate was not considered carcinogenic under the conditions of the study because the treatment did not alter the spontaneous tumor profile in rats (Manus et al., 1981; MRID# 00125233; satisfies Guidelines 870.4300).

In a chronic toxicity study, groups of beagle dogs (6/sex/group) were administered phorate via capsules at doses of 0, 0.005, 0.01, 0.05, or 0.25 mg/kg/day for one year. Compound related effects included slight body tremors in high dose males and females and marginal inhibition of body weight gain in high dose males. The systemic NOAEL was 0.05 mg/kg/day and the LOAEL was 0.25 mg/kg/day based on body tremors in males and females and inhibited body weight gains in males. The NOAEL for plasma ChE inhibition was 0.01 mg/kg/day while the LOAEL was 0.05 mg/kg/day for both sexes. The NOAEL for RBC or brain ChE inhibition was
0.05 mg/kg/day while the LOAEL was 0.25 mg/kg/day for both sexes (Shellenberger and Tegeris, 1987; MRID# 40174527; satisfies Guideline 870.4100).

In a carcinogenicity study, groups of CD-1 mice (50/sex/group) received phorate at a dietary concentration of 0, 1, 3, or 6 ppm (equivalent to 0, 0.15, 0.45, and 0.9 mg/kg/day) for 78 weeks. There were no consistent toxic signs or any non-neoplastic pathologic findings related to test compound administration. The NOAEL was 0.45 mg/kg/day and the LOAEL was 0.9 mg/kg/day based on a slight decrease in weight gain in females in the first 25 weeks. The dose level tested was considered adequate for carcinogenicity testing based on the results of the range finding study. The treatment did not alter the spontaneous tumor profile in this strain of mice (Manus et al. 1981; MRID# 00124845; satisfies Guideline 870.4200).

d. Developmental Toxicity

Technical phorate in corn oil was administered by oral intubation to pregnant rats (23 female/group) from day 6 to day 15 of gestation at dosages of 0, 0.125, 0.25, or 0.5 mg/kg/day. No developmental effects were observed in this study at any dosage. The NOAEL for both maternal toxicity and developmental toxicity was 0.25 mg/kg/day. The LOAEL for each was 0.5 mg/kg/day in which dams exhibited increased mortality, convulsions, and hypothermia while the fetuses showed enlarged hearts. The enlargement of the heart was considered to be a physiologic effect as a result of increased

acetylcholine, producing excessive stimulation of the myocardium with ensuing enlargement (Beliles, 1979; MRID# 00122775; satisfies Guideline 870.3700). Groups of pregnant rabbits (20/group) were administered 0, 0.15, 0.5, 0.9 or 1.2 mg/kg/day of phorate by gavage on days 6-18 of gestation. The maternal NOAEL was 0.15 mg/kg/day and the maternal LOAEL was 0.5 g/kg/day based on body weight loss and increased mortality. The developmental NOAEL was 1.2 mg/kg/day (the highest dose tested). No developmental effects were observed (Schroeder, 1987; MRID# 40174528; satisfies Guideline 870.3700).

In a developmental toxicity study, pregnant Crl:CD®BR rats (24-25/dose) received oral administration of Phorate (92.1%) in corn oil at dose levels of 0, 0.1, 0.2, 0.3 or 0.4 mg/kg/day from days 6 through 15 of gestation. For maternal toxicity, the NOAEL was 0.3 mg/kg/day and the LOAEL was 0.4 mg/kg/day, based on increased mortality, clinical signs indicative of neurotoxicity, decreases in body weight and body weight gain and food consumption and gross pathology. Developmental toxicity was manifested as decreased fetal weights and increased incidence of skeletal variations (delayed ossification of the sternum and pelvis). For developmental toxicity, the NOAEL was 0.3 mg/kg/day and LOAEL was 0.4 mg/kg/day (Lochry, 1990; MRID No. 44422301; satisfies Guideline 870.3700).

e. Reproductive Toxicity

There was a 3-generation reproductive study in mice (1965; MRID# 00092853) submitted to the Agency. In this study, technical phorate was administered in the diet to mice at dietary levels of 0, 0.6, 1.5 or 3.0 ppm (equivalent to 0, 0.09, 0.23, and 0.45 mg/kg/day, respectively). Compound administration was initiated 7 weeks before the first mating. The study involved 3 generation with 2 litters (a and b) per generation. The only apparent indications of reproductive toxicity were slight reductions in the lactation and viability indices in the F1b at the highest dose level.
The NOAEL was estimated to be 1.5 ppm (0.23 mg/kg/day) and the LOAEL was 3.0 ppm (0.45 mg/kg/day) based on effects on viability and lactation indices. This 3-generation reproduction study was down-graded from core minimum to unacceptable by the HED/RfD Peer Review Committee (December 30, 1993).

In a two-generation reproduction study, groups of male and female Sprague-Dawley rats (25/sex) were fed diets containing Phorate (92.1%) at dose levels of 0, 1, 2, 4, or 6 ppm (0, 0.087, 0.176, 0.359 or 0.603 mg/kg/day for males and 0, 0.103, 0.210, 0.420 or 0.727 mg/kg/day for females) for two successive generations. For parental systemic toxicity, the NOAEL was 0.2 mg/kg/day and the LOAEL was 0.4 mg/kg/day based on clinical signs (tremors) and inhibitions of plasma and brain cholinesterase activity (F1 females only). For offspring toxicity, the NOAEL was 0.2 mg/kg/day and the LOAEL was 0.4 mg/kg/day based on decreased pup survival and pup body weight. The decrease in pup survival was seen during early lactation and the decrease in pupbody weights was seen during the later part of lactation (Schroeder, 1991; MRID No. 44422302;satisfies Guideline 870.3800).

f. Mutagenicity

Sufficient data are available to satisfy data requirements for mutagenicity testing. Technical phorate did not induce a genotoxic response in any of the tests listed below.

- Gene mutation assays -
In an Ames assay, phorate was negative at dosages up to 1000 :g/plate with Salmonella typhimurium strains TA100, TA 1535, TA 1537, and TA 1538 in the presence and absence of metabolic activation (Simmon et al., 1977; MRID# 00124901). A test for reverse mutation in Escherichia coli was negative at dosages up to 1000 :g/plate in the presence and absence of metabolic activation (Simmon et al., 1977; MRID# 00124901). Phorate did not induce gene mutations at the HGPRT locus in cultured Chinese hamster ovary (CHO) cells at dosages up to 100 nL/mL with and without metabolic activation (Thilagar et al., 1985; MRID# 00151633).

- Chromosomal aberration assays-
A dominant lethal test in mice was negative at dosages up to 20 mg/kg in the diet (Simmon et al.,1977; MRID# 00124901). A chromosomal aberrations test was negative in mammalian (rats) bone marrow cells at ip (intraperitoneal) dosages up to 2.5 and 1.5 mg/kg in males and females, respectively (Ivett, 1986; MRID# 00155597).

- Other genotoxic effects studies –

Negative in mitotic recombination assay with Saccharomyces cerevisiae D3 at a concentration of 5% with and without metabolic activation (Simmon et al., 1977; MRID# 00124901).

Preferential toxicity assays in DNA repair-proficient and -deficient strains of Escherichia coli and Bacillus subtillis at a level of 1000 :g/plate were negative (Simmon et al., 1977; MRID# 00124901).

Preferential toxicity assays in DNA repair-proficient and -deficient strains of Bacillus subtilis (strain H17 and M45, respectively) at 1000 :g/plate were negative (Simmon et al., 1977; MRID# 00124901).

Unscheduled DNA synthesis (UDS) assay in human fibroblasts (WI-38 cells) at concentrations up to 10-3 M (Mol/L) did not show mutagenic response (Simmon et al., 1977; MRID# 00124901).

g. Metabolism

Data are available from rat metabolism studies in males and females. A single oral dose of 0.8mg/kg 14C-phorate was administered to male rats. The chemical was readily absorbed and excreted, with approximately 77.2% of the total administered 14C in the urine and 11.7% in the feces within 24 hours. Less than 1% of the total radioactivity was found in tissues (highest level in blood) at 24 hours. Ten metabolites were present in the

urine. Two non-phosphorylated metabolites, ethyl (methyl sulfinyl) methyl-sulfone and (ethyl sulfonyl)(methyl-sulfonyl) methane, comprised approximately 71% of the radioactivity present in the urine. About 9% and 10% of the urinary 14C was associated with (O,O-diethyl S-(ethyl sulfonyl) methyl phosphorothioic acid and [(ethyl sulfinyl) methyl, methyl sulfone], respectively. Unchanged parent compound accounted for only 0.5% of the recovered urinary 14C and the remaining four phosphorylated compounds plus one unidentified metabolite together comprised less than 10% of the urinary radioactivity. These metabolites were formed following cleavage of the sulfurphosphorus bond associated with the carbon chain in phorate, from methylation of the liberated thiol group, and from oxidation of the resulting sulfide to sulfoxide and sulfone (Hussain, 1987; MRID# 40291601).

Female rats showed a comparable pathway to that described for males (Miller and Wu, 1991; MRID# 41803803).

h. Neurotoxicity

In a delayed neurotoxicity study, 14.2 mg/kg (LD50 dose) of phorate was administered orally tohens followed by a 21-day interval and a second administration at the same dosage level. Phorate did not cause neurological changes indicative of delayed neurotoxicity (US EPA, 1988; Fletcher, 1984; MRID# 00152640; Guideline 870.6100).

In an acute neurotoxicity study, 4 groups of 7 week old Sprague-Dawley CD® rats (20/sex/group) were given a single oral dose (by gavage) of phorate (91.8% a.i.) in corn oil at doses of 0, 0.25, 0.50, or 1.0 mg/kg of body weight. The first indication of a compound-related effect (miosis) detected by the FOB was reported at the 0.5 mg/kg (2/10 males and 2/10 females) and 1.0 mg/kg (2/10 males and 5/10 females) doses approximately 4-5 hours after exposure to the test substance (time of peak effect). In addition to miosis, one female at the 1.0 mg/kg dose level showed evidence of slight tremors, fasciculations, slightly impaired locomotion, and splayed/dragging hindlimbs. Another female in this dose group also exhibited moderate tremors. These symptoms resolved within the first week and were no longer evident at the Day 8 observation period. Significant cholinesterase activity inhibition in plasma, red blood cells, and the brain was seen in both males and females at the 1.0 mg/kg dose level. A small but statistically significant decrease in brain cholinesterase activity (6%) was also observed in males at the 0.5 mg/kg dose level. Under the conditions of this study, the LOAEL is established at 0.50 mg/kg/day based on the evidence of miosis and of statistically significant brain
acetylcholinesterase inhibition in males. The NOAEL is 0.25 mg/kg/day. (Mandella 1998; MRID# 44719901, satisfies Guideline 870.6200).

No subchronic neurotoxicity study on phorate is available. Since phorate is an organophosphate, a subchronic neurotoxicity study is required as confirmatory data to support the re-registration of this chemical. The Agency has received a developmental toxicity study in rats and a 2-generation reproduction toxicity study in rats that do not show increased susceptibility for infants and children exposed to phorate. In addition,

these studies do not demonstrate any findings indicative of effects on the developing nervous system. Although this would provide support for not requiring a developmental neurotoxicity, it was noted that histopathological evaluation of perfused tissue in rats was not available in the data base. Due to concerns regarding the potency of this chemical, and in the absence of this histopathological data, the Hazard Identification Assessment Review
Committee (HIARC), at the February 3, 1998 meeting decided to place the requirement for a developmental neurotoxicity study under **reserve** status pending receipt and evaluation of the acute and subchronic neurotoxicity studies.

i. Dermal Absorption

No dermal absorption studies are available. The dermal absorption is considered to be 100% for the purposes of risk assessment because the chemical is very acutely toxic (Tox Category I) by either oral or dermal administration (Toxicology Endpoint Selection Committee meeting of 1/23/96, and confirmed by the HIARC 12/3/98).

j. Other Toxicological Considerations

No data are available on the eye effects of phorate in specialized acute and subchronic studies. The Toxicology Chapter of the Registration Standard for Phorate (December, 1988) indicated that additional specialized studies are required to determine the potential for phorate to induce adverse ocular effects in acute and subchronic studies in rats and a six month study in dogs, rabbits, or monkeys. The Agency has determined that these studies are no longer required, based on the recommendation of the FIFRA Scientific Advisory Panel (SAP) that these studies should not be routinely required for organophosphate pesticides (March 1997).

Phorate sulfoxide (a phorate metabolite) was administered to rats (35/sex) at dietary levels of 0, 0.32, 0.8 or 2.0 ppm (equivalent to 0, 0.016, 0.04, and 0.10 mg/kg, respectively) for 90 days. Sporadic inhibition of RBC and plasma ChE activity was observed in females at the 0.8 ppm level. At 2.0 ppm, RBC, plasma, and brain ChE activities were inhibited in females while only marginal inhibition of RBC and plasma ChE activity was noted in males. No other dosagerelated adverse effects were reported in this study. The NOAEL was 0.32 ppm (0.016 mg/kg) and the LOAEL was 0.8 ppm (0.04 mg/kg) based on inhibition of plasma and RBC ChE
activities (Hutchison at al., 1968; MRID# 00092912).

(Ethylsulfonyl) (methylsulfonyl) methane, a phorate metabolite, has an acute oral LD50 value of greater than 5000 mg/kg. In addition, this phorate metabolite does not have the structuralproperties of a cholinesterase inhibitor. Therefore, this phorate metabolite is not expected to bean acute toxicological concern (Lowe and Fischer, 1987, MRID# 40174526).

Phorate can be metabolized to more potent anticholinesterase compounds through oxidative desulfuration and/or sulfide oxidation. The oxidation products include the

sulfoxide and sulfone derivatives of phorate and a phorate oxygen analogue. Findings of the rat metabolism study showed that the oxidized, phosphorylated products represented minor proportions of the phorate metabolites measured in tissues, feces, and urine. Although the phorate sulfoxide metabolite appears to be slightly more toxic than the parent (as demonstrated above in the 90 day rat study with a Phorate) both compounds are very toxic and there is not much difference in their relative toxicity. For this reason, all of the data supporting phorate are adequate to support the metabolites which also inhibit cholinesterase. The Agency reserves the option to require additional toxicity studies with the oxidized metabolites if significant residue levels are detected.

2. Dose Response Assessment

Table 2. Summary of Toxicological Endpoints for Phorate

EXPOSURE SCENARIO	DOSE (mg/kg/day)	ENDPOINT	STUDY	MOE REQUIRED
Acute Dietary	NOAEL=0.25	Miosis	Acute Neurotoxicity - Rat	300
Chronic Dietary	NOAEL=0.05	Inhibition of RBC and brain cholinesterase activity	Chronic Toxicity - Dog	300
Short-Term (Dermal) a	Oral NOAEL=0.05	Inhibition of RBC and brain cholinesterase activity	Chronic Toxicity - Dog	300
Intermediate-Term (Dermal) a	Oral NOAEL=0.05	Inhibition of RBC and brain cholinesterase activity	Chronic Toxicity - Dog	300
Long-Term (Dermal) a	Oral NOAEL=0.05	Inhibition of RBC and brain cholinesterase activity	Chronic Toxicity - Dog	300
Inhalation (Any time period)a	Oral NOAEL=0.05	Inhibition of RBC and brain cholinesterase activity	Chronic Toxicity - Dog	300

a = Appropriate route-to-route extrapolations should be performed for these risk assessments [i.e., the dermal and inhalation exposure components using the appropriate absorption rates (100% default value for dermal and for inhalation) should be converted to equivalent oral doses and compared to the oral NOAEL).