# PHORATE

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## Explanation

Phorate is the International Organization for Standardization (ISO) approved name for phosphorothioic acid, *O*,-diethyl *S*-(ethyl thio)methyl ester, which is an organophosphate insecticide that inhibits acetylcholinesterase activity and is a systemic and contact insecticide and acaricide. Phorate was first evaluated by the Joint Meeting in 1977. In 1985, an acceptable daily intake (ADI) of 0–0.0002 mg/kg bw was established. Phorate was re-evaluated in 1994 when an ADI of 0–0.0005 mg/kg bw was established. In 1994, because it was reported in a limited study of metabolism in rats that <40% of the administered dose was excreted, the Meeting requested adequate studies on absorption, for review in 1996. Such studies were received and the ADI established previously was confirmed.

Since the 1994 JMPR, a study of acute neurotoxicity and a 13-week study of neurotoxicity in rats have been submitted. The present Meeting re-evaluated phorate within the periodic review programme of the Codex Committee on Pesticide Residues. The Meeting

considered new data that had not been reviewed previously and relevant data from previous evaluations.

Phorate is used against sucking and chewing insects, leafhoppers, leafminers, mites, some nematodes and rootworms, in order to protect a variety of crops, including corn, cotton, potatoes, tomatoes, sugar beets, edible beans, carrots, celery and peppers. Phorate is primarily formulated as granules to be applied at planting in a band or directly to the seed furrow. It is also used as a nematocide.

## Evaluation for acceptable daily intake

### 1. Biochemical aspects

#### *1.1 Absorption, distribution and excretion*

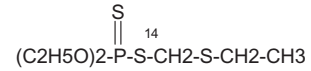
*Rats*

The 1994 review of studies of the metabolism of phorate indicated that male rats given $^{32}$P-labelled phorate as a single oral dose at 2 mg/kg bw excreted 35% of the administered radiolabel in the urine and 3.5% in the faeces within 144 h. Male rats given six oral doses of phorate at 1 mg/kg bw per day excreted 12% of the administered radiolabel in the urine and 6% in the faeces within 7 days. Brain, liver, and kidney tissues from the latter animals contained unidentified, largely unextractable residues (Bowman & Casida, 1958).

Male rats were given a single dose of $^{14}$C-labelled phorate (purity, >98%; specific activity, 40 µCi/mg; see Figure 1 for position of $^{14}$C) at a dose of approximately 0.8 mg/kg bw by gavage in corn oil. Most of the administered dose was excreted in the urine (77%) and faeces (12%) within the first 24 h after dosing. Ninety-seven percent of the administered dose was recovered in the urine, faeces and cage rinses within 192 h (8 days). Peak tissue concentrations of total radioactive residue were found 6 h after treatment: blood, 0.37 ppm; kidney, 0.29 ppm; liver, 0.24 ppm in liver; skin, 0.20 ppm; muscle, 0.14 ppm; and fat, 0.08 ppm. The levels of residue declined throughout the course of the study, and by 48 h the concentrations of residue in muscle, fat, and skin were all <0.01 ppm. By 192 h, the residues in liver were only 0.02 ppm and those in kidney were <0.01 ppm. Approximately 80%, 79%, 84%, and 69% of the total residue present in urine, kidney, muscle and liver, respectively, was composed of a nonphosphorylated series of metabolites arising from the cleavage of the sulfur–phosphorus bond, methylation of the liberated thiol group and oxidation of the resulting sulfide to sulfoxide and sulfone. The remaining residue consisted of the phosphorylated metabolites. This study demonstrated that rats given phorate at a dose of 0.8 mg/kg bw quickly metabolize the parent compound and rapidly excrete the bulk of the biotransformed products, primarily in the urine (Hussain, 1987).

$^{14}$C-Labelled phorate (purity, >98% specific activity, 40 µCi/mg; see Figure 1 for position of radiolabel) was rapidly absorbed and excreted by female rats given a single oral dose at 0.44 mg/kg bw by gavage in corn oil. The urine was the primary route of elimination, and accounted for 78% of the administered dose within 24 h. Faecal elimination accounted for

***Figure 1. Position of radiolabel in $^{14}$C-labelled phorate***



only 8% of the administered dose. Peak tissue levels of total radioactive residue were found after 6 h: blood, 0.168 ppm; kidney, 0.163 ppm; liver, 0.142 ppm; skin, 0.109 ppm; muscle, 0.100 ppm; and fat, 0.031 ppm. After 192 h (8 days), residues in the liver and kidney had depleted to only 0.008 and 0.010 ppm, respectively, while those in muscle, fat, skin, and blood were below the limit of detection of the assay by 48 h. After 192 h (8 days), 97% of the administered dose had been recovered in the urine, faeces and cage rinses. The results of this study indicate that phorate is rapidly absorbed and excreted by female rats. Greater than 94% of the administered dose was biotransformed to nonphosphorylated, non-toxicologically significant metabolites (Miller & Wu, 1990).

### *1.2 Biotransformation*

*Rats*

The 1994 review of studies of the metabolism of phorate indicated that the urine of male rats given phorate at a daily dose of 1 mg/kg bw contained 17% diethyl phosphoric acid, 80% *O,O*-diethylphosphorothioic acid, and 3% *O,O*-diethyl phosphorodithioic acid. When $^{32}$P-labelled phorate was incubated with prepared slices of rat liver, <1% of the radiolabelled compound was converted to hydrolysis products or unextractable residues. Phorate sulfoxide, phorate sulfone, phoratoxon sufoxide, and phoratoxon sulfone were formed (Bowman & Casida, 1958).

When a single dose of [$^{14}$C]phorate at 0.8 mg/kg bw was administered to male rats, the main urinary metabolites were the nonphosphorylated CL 180,298 (43%), CL 180,296 (28%), and CL 180,297 (9.6%) (see Figure 2 and Table 1 for identity of these metabolites). Phosphorylated metabolites (CL 18,061, CL 18,161, CL 18,162, CL 18,177, and CL 4,259) accounted for <15% of the recovered urinary metabolites, and the parent compound accounted for <1% of the administered dose. The main residues in liver, kidney, and muscle were also the nonphosphorylated metabolites, accounting for >68,79, and 83% of the tissue metabolites, respectively (Hussain, 1987).

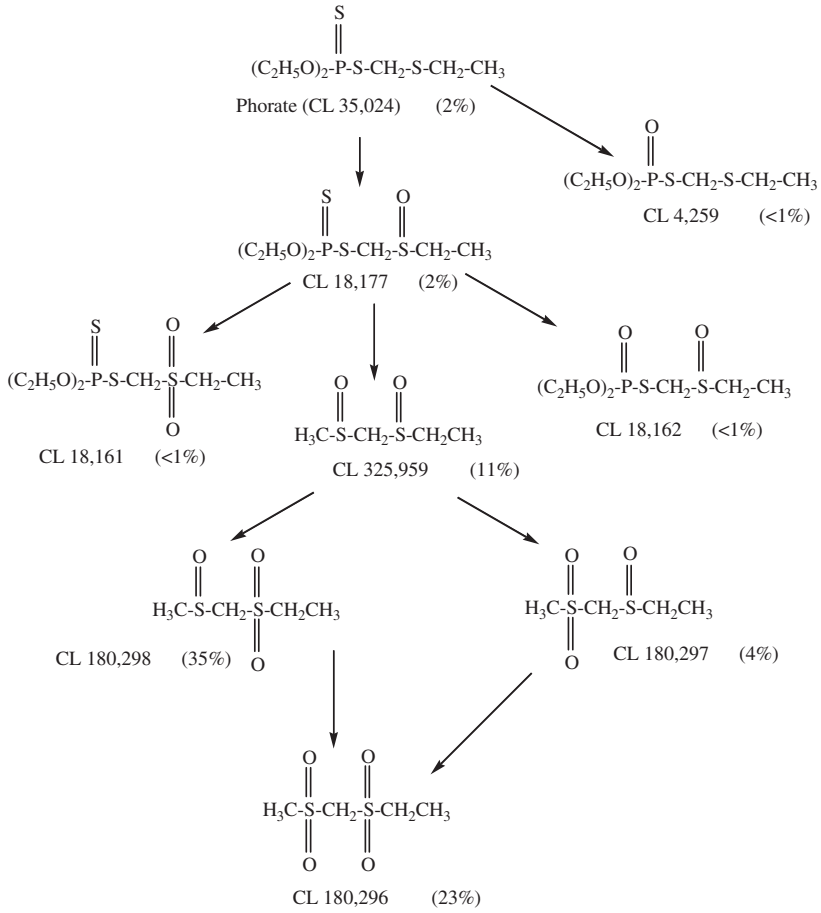
When female rats were given [$^{14}$C]phorate as a single dose at 0.44 mg/kg bw, the main urinary metabolites were the nonphosphorylated CL 180,298 (43%), CL 180,296 (24%), CL 325,959 (14%), and CL 180,297 (4.6%). No phosphorylated metabolites were identified in the tissues examined (liver, kidney and muscle). Two nonphosphorylated metabolites in the liver were identified as CL 180,286 (35%) and CL 180,298 <1%). Three metabolites in the muscle were identified as CL 180,296 (69.6%), CL 180,298 (7.0%) and CL 180,297

***Table 1. Identification codes and chemical names of phorate and its metabolites***

| Identification code | Chemical name |
|---|---|
| *Parent compound* | |
| Phorate (CL 35,024) | Phosphorodithioic acid, *O*,*O*-diethyl *S*-(ethylthio)methylester |
| *Metabolites* | |
| CL 18,162 | Phosphorothioic acid, *O*,*O*-diethyl *S*-(ethylsulfinyl)methylester |
| CL 18,177 | Phosphorodithioic acid, *O*,*O*-diethyl *S*-(ethylsulfinyl)methylester "phorate sulfoxide" |
| CL 4,259 | Phosphorothioic acid, *O*,*O*-diethyl *S*-(ethylthio)methylester |
| CL 18,161 | Phosphorodithioic acid, *O*,*O*-diethyl *S*-(ethylsulfonyl)methylester "phorate sulfone" |
| CL 180,298 | Sulfoxide, (ethylsulfonyl)methyl |
| CL 180,297 | Sulfoxide, ethyl(methylsulfonyl)methyl |
| CL 180,296 | Methane, (ethylsulfonyl)(methylsulfonyl)- |
| CL 352,959 | Methane, (ethylsulfinyl)(methylsulfinyl)- |

From Miller & Wu (1990)

***Figure 2. Proposed metabolic pathway of phorate in rats***




From Miller & Wu (1990)

(<1%). Two metabolites in the kidney were identified as CL 180,296 (22.4%) and CL 180,298 (1.7%). Similarly, no phosphorylated metabolites were identified in the urine. Faecal samples contained primarily the unchanged parent compound (33%) and the phosphorylated metabolites CL 18,177 (24%), CL 18,161 (8.8%), CL 18,162 (5.5%), and CL 4,259 (4.3%). The proposed metabolic pathway is presented in Figure 2, and the chemical names of the metabolites are given in Table 1 (Miller & Wu, 1990).

Phorate was rapidly absorbed and extensively detoxified by rats given a single oral dose by gavage. Urinary excretion was the principal route of elimination, accounting for approximately 80% of the administered dose within 24 h after dosing. Faecal excretion accounted for approximately 10% of the administered dose. After 192 h (8 days), essentially the entire administered dose had been eliminated by excretion.

The bulk of the administered dose (94%) was biotransformed to nonphosphorylated metabolites. The metabolic pathway for formation of these metabolites resulted from the cleavage of the phosphorus–sulfur bond, methylation of the liberated thiol group and oxidation of the resulting divalent sulfur moiety to the sulfoxide and sulfone. Thus, these studies demonstrated that phorate is rapidly absorbed and excreted and the accumulation of any toxic residue is not a concern.

### *1.3 Effects on enzymes and other biochemical parameters*

Male Swiss albino mice were given phorate at a dose of 6728.5 mg/m$^3$ administered by inhalation using a whole-body inhalation chamber. Biochemical parameters were measured after 2, 4, 6, 8, 10, and 12 weeks of treatment. A dose-dependent significant decrease in pseudocholinesterase activity was observed throughout the experimental period. Concentration of haemoglobin, and erythrocyte count and erythrocyte volume fraction were significantly decreased from week 6 until the end of the experiment. During week 2, a significant increase in the total leukocyte count was observed, which was associated with an increase in monocyte and neutrophil counts. Subsequently, a significant decrease in counts was observed. The lungs of exposed animals showed a varying degree of bronchopneumonia and emphysema. After week 4, the animals slowly acclimatized and showed signs of recovery in haemoglobin and erythrocytes, while the leukocyte count decreased continuously. The sudy indicated that exposure to phorate leads to emphysematous changes and increased leukocyte count (Morowati, 1999).

The relative contribution of the cytochrome P450 (CYP)-dependent mono-oxygenase system and the flavo-containing monooxygenase in the microsomal oxidation of phorate were investigated in mice treated with phenobarbital, piperonyl butoxide, acetone, or hydrocortisone. Treatment with piperonyl butoxide produced a distinct biphasic effect (initial inhibition and subsequent induction) on the activities of several hepatic enzymes, including sulfoxidation of phorate. The relative contribution by CYP to phorate sulfoxidation decreased from 76% in the controls to 58% at 2 h after treatment and increased to 89% at 12 h. Treatment with hydrocortisone caused an increase in flavo-containing monooxygenase activity in the liver, but not in lung microsomes. Administration of acetone caused an increase in benzphetamine *N*-demethylation and *p*-nitrophenol hydroxylation in the liver, but no change in phorate sulfoxidase activity was observed, nor were the relative contributions attributable to CYP and flavo-containing monooxygenase altered. Treatment with phenobarbital produced a large increase in both benzphetamine *N*-dimethylase and phorate sulfoxidase activity in liver microsomes, with the percentage of the latter being increased due to CYP from 76% in the controls to 85% in the livers of treated rats (Kinsler, 1990).

## 2. Toxicological studies

### *2.1 Acute toxicity*

#### *(a) General toxicity*

The results of studies of acute toxicity with technical-grade phorate are summarized in Table 2.

#### *(b) Oral toxicity*

The acute oral toxicity of technical-grade phorate was investigated in groups of male or female rats or mice (Table 2). The animals were observed for mortality and signs of toxicity for 14 days after administration. All the animals that received toxic or lethal doses showed typical clinical signs of cholinergic toxicity, such as salivation, lacrimation, exophthalmos, muscle fasciculation and excessive urination and defecation.

#### *(c) Dermal toxicity*

The acute dermal toxicity of technical-grade phorate was investigated in 10 Sprague-Dawley rats given single doses by dermal application (Newell & Dilley, 1978). Dilutions

***Table 2. Results of studies of acute toxicity with technical-grade phorate***

| Species | Strain | Sex | Route and vehicle | $LD_{50}$ (mg/kg bw) | $LC_{50}$ (mg/l of air) | Reference |
|---|---|---|---|---|---|---|
| Mouse | NS | Male | Oral, in propylene glycol | 2.25 | — | Gaines (1969) |
| Mouse | NS | NS | Oral | 11.00 | — | Blinn (1982) |
| Mouse | NS | NS | Intraperitoneal | 3.00 | — | Blinn (1982) |
| Rat | Sherman | Male | Oral, in peanut oil | 2.30 | — | Gaines (1969) |
| Rat | Sherman | Female | Oral, in peanut oil | 1.10 | — | Gaines (1969) |
| Rat | Sprague-Dawley | Male | Oral, in propylene glycol | 3.7 | — | Newell & Dilley (1978) |
| Rat | Sprague-Dawley | Female | Oral, in propylene glycol | 1.40 | — | Newell & Dilley (1978) |
| Rat | Sprague-Dawley | Male | Oral | 1.9–10 | — | Blinn (1982) |
| Rat | NS | NS | Dermal | 3.0 | — | Blinn (1982) |
| Rat | Sprague-Dawley | Female | Dermal, in propylene glycol | 3.90 | — | Newell & Dilley (1978) |
| Rat | Sprague-Dawley | Male | Dermal, in propylene glycol | 9.30 | — | Newell & Dilley (1978) |
| Rat | Sherman | Male | Dermal, in xylene | 6.20 | — | Gaines (1969) |
| Rat | Sherman | Female | Dermal, in xylene | 2.50 | — | Gaines (1969) |
| Rat | Sprague-Dawley | Female | Intravenous | 1.20 | — | Newell & Dilley (1978) |
| Rat | Sprague-Dawley | Male | Intravenous | 2.20 | — | Newell & Dilley (1978) |
| Rat | Sprague-Dawley | Female | Inhalation | — | 0.011 (1 h) | Newell & Dilley (1978) |
| Rat | Sprague-Dawley | Male | Inhalation | — | 0.06 (1 h) | Newell & Dilley (1978) |

NS, strains not specified

of the test substance were made in propylene glycol. The animals were observed for mortality and signs of toxicity for 14 days after administration. All the animals that received toxic or lethal doses showed typical clinical signs of cholinergic toxicity, such as salivation, lacrimation, exophthalmos, muscle fasciculation and excessive urination and defecation.

The dermal $LD_{50}$ values for phorate were 9.3 (range, 7.9–11.0) mg/kg bw for males and 3.9 (range, 3.4–4.4) mg/kg bw for females.

*(d) Exposure by inhalation*

To determine the acute toxicity of technical-grade phorate administered by inhalation, groups of 10 male or female Sprague-Dawley rats were treated with an aerosol of phorate (technical grade, not diluted) by whole-body exposure (Newell & Dilley, 1978). The animals were observed for mortality and signs of toxicity during exposure and for 14 days after administration. All the animals that received toxic or lethal doses showed typical clinical signs of cholinergic toxicity, such as salivation, lacrimation, exophthalmos, muscle fasciculation and excessive urination and defecation.

The median lethal concentration ($LC_{50}$) values for an exposure of 1 h were calculated to be 0.06 (range, 0.052–0.069) mg/l of air for males and 0.011 (range, 0.007–0.015) mg/l of air for females.

*(e) Dermal and ocular irritation and dermal sensitization*

Since technical-grade phorate is highly toxic by dermal contact, dermal irritation potential could not be determined.

Since technical-grade phorate is highly toxic by dermal contact, ocular irritation potential could not be determined.

Because of the marked acute toxicity of technical-grade phorate by dermal contact, a study of dermal sensitization was not performed.

### *2.2 Short-term studies of toxicity*

*Mice*

In a study that complied with the principles of good laboratory practice (GLP) and was certified for quality assurance (QA), groups of 20 male and 20 female Crl:CD1®(ICR)BR (outbred Swiss albino mice) were given access ad libitum to diets containing technical-grade phorate (purity, 92.1%) at a concentration of 0, 1, 3, or 6 ppm for 13 weeks. Terminal sacrifice was performed during week 14. Survival, clinical signs, body weights, food consumption, findings after necropsy for an unscheduled death, and terminal studies of clinical pathology (plasma, erythrocyte, and brain cholinesterase activities) were evaluated. Histopathological examinations were not performed.

The mean daily intake of phorate is summarized in Table 3.

There were no toxic effects evident in any of the treated groups of mice in terms of survival rates (survival/adjusted survival was 100%), body weight and food consumption measurements, or clinical and necropsy observations. At 6 ppm, one male convulsed for about 20 s when placed in the weighing pan at week 13. Similar findings occasionally occur in untreated mice in this laboratory and are thought to represent spontaneous epileptic seizures. The relationship, if any, of this isolated finding to treatment in this study is not known.

Terminal studies of clinical pathology showed inhibition of cholinesterase activities in females at 1 ppm and in males and females at 3 and 6 ppm. Apparent effects were greater on the plasma than on erythrocytes or the brain. Plasma cholinesterase activities were below those of controls for females at 1 ppm (15%), males (47%) and females (61%) at 3 ppm and males (82%) and females (88%) at 6 ppm. Erythrocyte cholinesterase activities were decreased by 17% for females at 3 ppm and by 50% and 61% for males and females at 6 ppm. These blood values were significantly decreased (except for the erythrocyte cholinesterase activity of females at 3 ppm) as were brain cholinesterase activities at the two highest dietary concentrations—in males and females at 3 ppm (left half of the brain showed a 12% decrease for males; right and left brain halves showed decreases of 9% and 13% for females) and in males and females at 6 ppm (decreases of 49% and 50% in males, and 53% and 54% in females, for left and right brain halves, respectively).

In conclusion, under the conditions of this study, administration of diets containing phorate at a concentration of 1, 3, or 6 ppm to Swiss albino mice for 13 weeks produced dose-related inhibition of cholinesterase activity, but no other apparent signs of toxicity. Effects on plasma cholinesterase activity exceeded those observed for erythrocyte or brain cholinesterase activity.

***Table 3. Mean daily substance intake in mice fed diets containing phorate for 13 weeks***

| Dietary concentration (ppm) | Mean daily intake (mg/kg bw) | |
|---|---|---|
| | Males | Females |
| 1 | 0.18 | 0.23 |
| 3 | 0.55 | 0.67 |
| 6 | 1.10 | 1.38 |

From Trutter (1990)

On the basis of inhibition of erythrocyte and brain cholinesterase, the lowest-observed-adverse-effect level (LOAEL) was 6 ppm (equal to an intake of 1.10 mg/kg bw per day), supporting a no-observed-adverse-effect level (NOAEL) of 3 ppm (equal to 0.55 mg/kg bw per day). The 9% and 17% decreases in erythrocyte and brain cholinesterase activities, respectively, at 3 ppm were not considered to be toxicologically significant (Trutter, 1990).

*Rats*

In a 13-week study, groups of 50 male and 50 female (control and groups B, C, D and E) or 25 male and 25 female (groups F and G) albino Carworth Farms rats were given access ad libitum to diets containing technical-grade phorate (purity, 92%) at a concentration 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 or 0.9 mg/kg bw per day respectively) for 13 weeks.

Males and females at 12 and 18 ppm showed severe excitability, intermittent tremors and ataxia, which resulted in the death of 50% of the animals at 12 ppm and no survivors at 18 ppm. Occasional episodes of excitability, intermittent tremors were noted in females at 6 ppm. In all groups receiving phorate at ≤6 ppm, survival, body-weight gain, food consumption and liver and kidney weights were within normal limits.

Erythrocyte cholinesterase activity was inhibited (by approximately 30%) in females at 2 ppm, while at 6 ppm, plasma, erythrocyte and brain cholinesterase activities were inhibited in both sexes (brain cholinesterase by approximately 85% in females).

No adverse effects were observed at gross necropsy or histopathological examination.

The NOAEL was 2 ppm (equal to 0.1 mg/kg bw per day) on the basis of inhibition of brain acetylcholinesterase activity at 6 ppm (equal to 0.3 mg/kg bw per day) (Tusing et al., 1956b).

*Dogs*

Groups of two male and one female mongrel dogs received capsules containing technical-grade phorate (purity, 92%; in corn oil) at a dose of 0, 0.01, 0.05, 0.25 or 1.25 mg/kg bw per day, 6 days per week for 15 weeks. Two males received a single dose of 2.5 mg/kg bw per day. Plasma and erythrocyte cholinesterase activities were measured weekly; brain acetylcholinesterase activity was not measured. At 0.05 mg/kg bw per day, plasma cholinesterase activity was significantly depressed. Erythrocyte cholinesterase activity at 0.05 mg/kg bw per day was not affected during the first 12 weeks of the study, but was depressed slightly, not significantly, during the last 3 weeks of the study. Significant decreases in plasma and erythrocyte acetylcholinesterase activity were observed at 0.25 mg/kg bw per day; total inhibition of plasma cholinesterase activity and a significant reduction in erythrocyte cholinesterase activity were noted at 1.25 mg/kg bw per day. All the dogs receiving phorate as a single dose at 2.5 mg/kg bw per day died within 3–4 h; cholinesterase activity was not determined. No signs of systemic toxicity and no adverse effects on haematological, clinical chemistry or urine analysis parameters were observed in dogs at 0.01 and 0.05 mg/kg bw per day. Histopathological examination revealed no consistent treatment-related findings.

The NOAEL was 0.05 mg/kg bw per day on the basis of inhibition of erythrocyte cholinesterase activity at higher doses (Tusing et al., 1956a).

Groups of three male and three female beagle dogs were fed diets containing technical-grade phorate at a concentration of 0, 0.5 or 1.0 ppm, equivalent to 0.012 or 0.025 mg/kg bw per day, for 6 weeks. Cholinesterase activity in plasma and erythrocytes was determined before study initiation and every 2 weeks during the study. No significant differences in plasma and erythrocyte cholinesterase activities were noted between the treated and control animals (Kay & Calandra, 1961).

In a 14-day study designed to evaluate the toxicity of technical-grade phorate administered orally, five groups (groups 2 to 6) of two male and two female beagle dogs received capsules containing phorate (purity, 92.1%) at a dose of 0.01, 0.05, 0.1, 0.25 or 0.5 mg/kg bw per day for 14 consecutive days. A control group (group l, comprising three males and three females) was given capsules containing corn oil. Groups 4 to 6 began the study 1 week after groups 1 to 3. The dogs were observed for mortality and signs of pharmacotoxicity four times daily. Food consumption was measured and recorded daily. Body weights were recorded on days 1 and 8, and at terminal sacrifice. Blood biochemical and haematological parameters were determined for each dog during the pre-test period and before study termination. Special assays for erythrocyte and plasma cholinesterase activities were performed twice for each dog during the pre-test period and after 3, 7, 10 and 14 doses of the test substance. Brain acetylcholinesterase activity was determined in the cerebellum and cerebrum of each dog after termination of the study. All surviving animals were necropsied at termination.

Excessive salivation and tremors seen in animals at the highest dose were probably related to administration of the test substance, while salivation seen at 0.05 mg/kg bw per day was probably a random occurrence. A slight decrease in body-weight gain in animals at the highest dose was probably related to administration of phorate. No definitive treatment-related effects were observed on food consumption, haematology, or organ weights. Necropsy did not reveal any gross lesions in any of the treated dogs. A depression in serum protein values was observed at 0.5 mg/kg bw per day in both males and females. Plasma cholinesterase activity was inhibited at doses of ≥0.05 mg/kg bw per day in males and at ≥0.1 mg/kg bw per day in females. Cholinesterase activity in the brain (cerebellum) was inhibited (by 31–69%) in males and females at doses of ≥0.1 mg/kg bw per day. Erythrocyte cholinesterase activity was slightly inhibited (by approximately 25%) in males and females at the highest dose tested, 0.5 mg/kg bw per day. These findings were considered to be treatment-related.

The NOAEL was 0.05 mg/kg bw per day on the basis of inhibition of brain acetylcholinesterase activity (Piccirillo et al., 1987).

*Dogs*

In a 1-year study of oral toxicity, groups of purebred beagle dogs (aged 6 months) were given capsules containing technical-grade phorate (purity, 92.1%) at a dose of 0, 0.005, 0.01, 0.05 or 0.25 mg/kg bw per day. The treated groups comprised six animals of each sex per group while the control group comprised eight animals of each sex. All animals were observed twice daily for overt signs of toxicity; detailed examinations were conducted weekly. Body weights were recorded before testing, at the start of the dosing period, weekly thereafter throughout the study, and at terminal sacrifice. Food consumption was determined daily throughout the study. Blood biochemical, haematological and urine analysis parameters and plasma and erythrocyte cholinesterase activities were determined before testing, at

6 weeks, 3, 6 months and at study termination. Acetylcholinesterase activities in the cerebrum and cerebellum were determined after termination of the study.

Clinical signs indicative of cholinergic toxicity (mild tremors) were seen occasionally in males and females at the highest dose (0.25 mg/kg bw per day). One male at 0.01 mg/kg bw per day was sacrificed in a moribund condition during week 24, but this was considered to be incidental to treatment. All other animals survived to the terminal necropsy with no consistent clinical signs that could be attributed to the administration of phorate. Mean body weights of males at the highest dose were consistently lower than those of the controls, but the differences were not statistically significant; mean body weights of females at all doses were similar to those of the controls throughout the study. Food consumption of treated males and females at all doses were similar to those of the controls throughout the study. Ophthalmological examinations revealed no treatment-related effects. Examinations of haematology, clinical chemistry parameters and urine analysis revealed no biologically significant changes.

At 0.01 mg/kg bw per day, there were marginal effects on plasma cholinesterase activity in males and females. However, statistically significant inhibition of plasma cholinesterase activity (25–80%) was observed in males and females at doses of 0.05 and 0.25 mg/kg bw per day. The highest dose, 0.25 mg/kg bw per day, resulted in reduced erythrocyte (20% inhibition) and brain cholinesterase (43–54% inhibition) activities in males and females.

The overall NOAEL was 0.05 mg/kg bw per day on the basis of decreased body weight, significant inhibition of erythrocyte and brain acetylcholinesterase activity and clinical signs consistent with cholinergic toxicity at the highest dose of 0.25 mg/kg bw per day (Shellenberger & Tegeris, 1987).

### *2.3 Long-term studies of toxicity and carcinogenicity*

*Mice*

The long-term toxicity and oncogenic potential of technical-grade phorate was evaluated in a 18-month study in mice. Groups of 50 male and 50 female CD1 outbred Swiss albino mice were given diets containing phorate (purity, 91.7%) at a concentration of 0, 1, 3 or 6 ppm (equal to 0, 0.15, 0.45 and 0.9 mg/kg bw per day). The animals were observed before the start of the study and at least daily thereafter for general physical appearance, mortality and signs of toxicity. Each animal was removed from the cage and examined weekly in detail for clinical signs, including palpation for masses. Body weight was measured before the start of the study; body weights and food consumption were subsequently obtained at weekly intervals through week 13, biweekly for weeks 15–25 and monthly until termination. Animals found dead or moribund and that were sacrificed during the study were necropsied and a spectrum of the tissues were preserved in 10% formalin for future evaluation. At 18 months, all remaining animals were killed and a gross necropsy was performed on each animal and tissues were preserved in 10% buffered formalin. Tissues saved from all animals were examined histopathologically.

During the course of the study, 91 mice died or were killed in a moribund condition, as summarized in Table 4.

***Table 4. Mortality to study termination in mice fed diets containing phorate for 18 months***

| Dietary concentration (ppm) | Cumulative mortality (%) | |
|---|---|---|
| | Males | Females |
| 0 | 5/50 (10%) | 13/50 (26%) |
| 1 | 7/50 (14%) | 13/50 (26%) |
| 3 | 11/50 (22%) | 16/50 (49)[a] (32% or 32.7%) |
| 6 | 9/50 (18%) | 17/50 (49)[a] (34% or 34.7) |

From Manus et al. (1981a)

[a] The totals shown above reflect situations in which mice were missing

None of the deaths were attributed to the effects of treatment as mortality was similarly distributed throughout control and treated groups.

Some clinical signs, such as tremors, hyperactivity and excessive salivation, occurred at a higher incidence and more frequently in animals fed with diets containing phorate at 6 ppm than in animals in the control groups.

The only treatment-related effect was a reduction in the mean body weight of females at 6 ppm throughout the study, with the differences (compared with controls) at 13 separate time-points being statistically significant. While there was a suggestion of a similar trend in males at 6 ppm, only at three early time-points were the differences statistically significant. Otherwise, the body weights of the treated animals were comparable to those of animals in the control group. Differences in mean body weight did not seem to be caused by reduced consumption of food. All treated animals appeared to eat less during the first 3 weeks and occasionally thereafter, but no consistent dose–response relationship was seen.

Gross pathological examination showed no changes that were significantly different from those in animals in the control groups, and histopathological examination revealed no alterations that were related to treatment. There was no significant dose-related increase in the incidence of any particular type of tumour, of animals with tumours, of animals with malignant tumours or of animals with multiple primary tumours. The NOAEL was 3 ppm (equal to 0.45 mg/kg bw per day) on the basis of decreased body weight and clinical signs of toxicity at 6 ppm (equal to 0.90 mg/kg bw per day) (Manus et al., 1981a).

*Rats*

In a long-term study of oral toxicity and potential carcinogenicity, groups of 50 male and 50 female Crl:COBS CD(SD)BR rats were given diets containing technical-grade phorate (purity, 91.7%) at a concentration of 0, 1, 3 or 6 ppm (equal to 0.05, 0.16 or 0.32 mg/kg bw per day in males and 0.07, 0.19 or 0.43 mg/kg bw per day in females) for 24 months. The parameters evaluated in this study were clinical signs, body weight, food consumption, haematology, clinical chemistry, urine analysis, and plasma, erythrocyte and brain cholinesterase activity; gross necropsy and histopathological evaluations were carried out at study termination.

The numbers of rats in each group that survived to the end of the study are summarized in Table 5.

***Table 5. Survival to study termination in rats fed diets containing phorate for 24 months***

| Dietary concentration (ppm) | No. of rats that survived to study termination (%) | |
|---|---|---|
| | Males | Females |
| 0 | 29/51 (57%) | 28/50 (56%) |
| 1 | 27/50 (54%) | 24/50 (48%) |
| 3 | 27/50 (54%) | 34/50 (68%) |
| 6 | 24/50 (48%) | 18/50 (36%) |

From Manus et al. (1981b)

The only clinical sign related to treatment was tremors induced by over-dosing (327% of all the intended doses) during week 9. Growth was depressed in females at 6 ppm during the first 26 weeks and again between weeks 74 and 102. Food consumption showed no consistent dose–response pattern. On haematological examination, clinical chemistry and urine analysis performed at 6, 12 and 24 months, the only notable findings were decreased values for erythrocyte counts, haemoglobin and erythrocyte volume fraction in females at the highest dose after 12 months.

Dose-related inhibition (>20%) of plasma cholinesterase activity was noted in males at 6 ppm at 12 months, in all treated males at 24 months and in females at both 3 and 6 ppm at all time-points (3, 6, 12 and 24 months). Erythrocyte acetylcholinesterase was not significantly depressed (<20%) at any time. The activity of brain acetylcholinesterase was reduced (>20%) in males at 6 ppm and in females at 3 and 6 ppm.

At sacrifice, females at 6 ppm had increased organ : body weight ratios with respect to the adrenals, brain, heart, liver and spleen. On gross pathological and histopathological examination, the only finding that could possibly be attributed to test substance administration was the slight increase in the incident of inflammation and epithelial hyperplasia of the forestomach in both males and females, but particularly in males at 6 ppm. Similar lesions were seen in animals in the control group, and have been reported to be a relatively common incidental finding in laboratory rats. There was no obvious trend in incidence of the lesions in the groups receiving the intermediate dose. For these reasons, the increased incidence of forestomach lesions in treated rats was considered to be attributable to random variation, or, at most, irritation of forestomach. There were no significant differences between control and treated groups with regard to incidence, type or time of appearance of tumours.

The NOAEL was 1 ppm (equal 0.07 mg/kg bw per day) on the basis of inhibition of brain acetylcholinesterase activity in females at 3 ppm (equal to 0.19 mg/kg bw per day) (Manus et al., 1981b).

### *2.4 Genotoxicity*

The results of studies of genotoxicity with phorate are summarized in Table 6. Phorate was not found to be genotoxic in vitro or in vivo.

***Table 6. Results of studies of genotoxicity with phorate***

| End-point | Test object | Concentration or dose | Purity (%) | Result | Reference |
|---|---|---|---|---|---|
| *In vitro* | | | | | |
| Reverse mutation | *S. typhimurium* TA100, TA1535, TA1537, TA1538; *E. coli* WP2 | ≤1000 mg/plate | Technical grade | Negative[a] | Allen (1978) |
| Reverse mutation | *E. coli* p3478, W3110; *B. subtilis* | 1 mg (on filter disc) per plate | 85 | Negative[b] | Simmon et al. (1977) |
| Reverse mutation | Chinese hamster ovary cells, *Hprt* locus | 30, 40, 50, 80, or 100 nl/ml | 92.1 | Negative[b] | Thilagar & Kumarop (1985) |
| | | 5, 10, 12, 14, 16, or 18 nl/ml | | Negative[c] | |
| Mitotic recombination | *S. cerevisiae* D3 | 5% w/v for 4 h incubation before plating | 85 | Negative[a] | Simmon et al. (1977) |
| Unscheduled DNA synthesis | Human fibroblasts WI-38 | $\leq 1 \times 10^{-3}$ | 85 | Negative[a] | Simmon et al. (1977) |
| *In vivo* | | | | | |
| Chromosomal aberration | Male and female Sprague-Dawley rats killed after 6, 18, or 30 h | Males: 0 (corn oil), 0.25, 1.25, or 2.5 mg/kg bw per day; Females: 0, 0.13, 0.63, or 1.25 mg/kg bw per day | 92.1 | Negative | Ivett & Myhr (1986) |
| Dominant lethal mutation | Male mice | 0, 5, 10, or 20 mg/kg bw per day in diet for 7 weeks, weekly matings for 8 weeks | 85 | Negative | Simmon et al. (1977) |

[a] In the presence and absence of metabolic activation
[b] In the absence of metabolic activation
[c] In the presence of metabolic activation

***Table 7. Mean daily substance intake in mice fed diets containing phorate for 3 weeks***

| Dietary concentration (ppm) | Mean daily intake (mg/kg bw) | |
|---|---|---|
| | Males | Females |
| 0.6 | 0.12 | 0.13 |
| 1.5 | 0.30 | 0.33 |
| 3.0 | 0.54 | 0.64 |

From Morici et al. (1965)

## *2.5 Reproductive toxicity*

### *(a) Multigeneration study*

#### *Mice*

Groups of eight male and 16 female CF1 mice were fed diets containing technical-grade phorate (purity, 98.4%) at a concentration of 0, 0.6, 1.5 or 3 ppm for three generations, with two litters per generation. Food intake was measured only for the $F_0$ generation before the initial mating. Since animals were housed in pairs, no measurement of individual consumption was obtained. The mean intakes of phorate for the 3-week period are summarized in Table 7.

There were no dose-related effects on indices of fertility, gestation, viability or lactation during the study, but the lactation index was lowered in four of the six litters in the group of animals receiving phorate at 3 ppm, to below the value for control animals in the first mating of the $F_0$ generation, in both matings of the $F_1$ generation and in the second mating of the $F_2$ generations. Gross and microscopic examination of tissues revealed no treatment-related effects.

The NOAEL was 1.5 ppm (equal to 0.30 mg/kg bw per day for males and 0.33 mg/kg bw per day for females) on the basis of decreased lactation indices at 3 ppm (Morici et al., 1965).

*Rats*

In a study that complied with the principles of GLP and that was certified for QA, reproductive performance and fertility were observed through two generations of rats that were given diets containing phorate (purity, 92.1%) at a concentration of 1, 2, 4 or 6 ppm. Each parent generation ($P_1$ and $F_1$), consisted of groups of 25 male and 25 female COBS CD®(SD) rats, except for the $F_1$ generation at 6 ppm that consisted of 30 males and 30 females at study initiation. Also included was a diet control group of 25 males and 25 females. Animals of the $P_1$ generation received at least 60 days of treatment before initiation of mating to produce the first litters, while animals of the $F_1$ generation received at least 100 days treatment before initiation of mating. During these pre-mating periods of treatment, body weights and food consumption were recorded weekly. Animals of the parental generation received a detailed physical examination weekly throughout the study. Body weights of mated females were recorded during gestation and lactation, and food consumption was recorded during gestation.

Each parental generation was mated to produce two litters. Randomly selected offspring from the second litter ($F_{1b}$) of the $P_1$ generation were chosen to become the parents of the ensuing generation. Offspring not included in the selection procedure and offspring from the first litter of each generation ($F_1$, $F_{2a}$) and the $F_{2b}$ offspring were given a gross external examination and discarded. Additionally, randomly selected offspring (one of each sex per litter) from the second litters of the $P_1$ and $F_1$ generations ($F_{1b}$ and $F_{2b}$ litters, respectively) were sacrificed and given a gross postmortem examination (abnormal tissues were saved in 10% formalin). Animals of the $P_1$ and $F_1$ adult generations were sacrificed, given a gross postmortem evaluation, and pituitary glands and reproductive tissues/gross lesions were taken and preserved in 10% neutral buffered formalin. The eyes from all $P_1$ parental females, and the eyes plus the intra- and extra-ocular muscles and optic nerve from all the $F_1$ parental animals were preserved in 10% formalin. Reproductive tissues and pituitary glands were processed for histopathological evaluations for the $P_1$ and $F_1$ animals in the control group and the group receiving phorate at a dietary concentration of 6 ppm, and ocular tissues, including the intra- and extra-ocular muscles, retina and optic nerve, were evaluated for all animals in the control group and treated $F_1$ parental animals. Additionally, in the $F_1$ parental animals, plasma, erythrocyte and brain cholinesterase activities were determined for 10 randomly selected animals of each sex per group at terminal sacrifice. Ophthalmoscopic evaluations were conducted for females of the $P_1$ parental generation before scheduled sacrifice and for all animals of the $F_1$ parental generation at initiation of the pre-mating period of treatment and again several weeks before scheduled sacrifice.

Mean daily intakes of the test substance for the treated groups, calculated from data on mean weekly food consumption during the pre-mating periods, are summarized in Table 8.

Treatment with phorate at a dietary concentration of 1 or 2 ppm produced no adverse effects in animals of the parental generation ($P_1$, $F_1$) with regard to the following parameters: growth during the pre-mating period of treatment; food consumption; physical observations; maternal-weight gain during gestation/lactation; maternal food consumption during

***Table 8. Mean daily substance intake in a study of reproductive toxicity in rats fed diets containing phorate***

| Dietary concentration (ppm) | Mean daily intake (mg/kg bw) | | | |
|---|---|---|---|---|
| | $P_1$ | | $F_1$ | |
| | Males | Females | Males | Females |
| 1 | 0.086 | 0.101 | 0.088 | 0.105 |
| 2 | 0.171 | 0.197 | 0.181 | 0.205 |
| 4 | 0.347 | 0.401 | 0.370 | 0.439 |
| 6 | 0.523 | 0.622 | 0.683 | 0.831 |

From Schroeder (1991)

***Table 9. Differences in mean cholinesterase activity in $F_1$ parental rats fed diets containing phorate compared with controls***

| Dietary concentration (ppm) | Difference (%) in mean cholinesterase activity | | | | | |
|---|---|---|---|---|---|---|
| | Males | | | Females | | |
| | Plasma | Erythrocyte | Brain | Plasma | Erythrocyte | Brain |
| 1 | +20.8 | +1.3 | +3.6 | +3.3 | −2.7 | −1.2 |
| 2 | −2.2 | 0 | +2.4 | −19.2 | −1.4 | −17.4 |
| 4 | −25.2 | −6.3 | −14.5 | −74.4** | −4.1 | −59.3** |
| 6 | −40.3** | −10.1* | −39.8** | −95.8** | −11.0 | −82.6** |

From Schroeder (1991)
*$p > 0.05$; **$p > 0.01$; statistically significant differences from data for controls

gestation; reproductive performance/fertility indices; duration of gestation; and parturition. For each litter interval, pup weights, pup sex distribution indices, pup survival indices during lactation and pup external examinations indicated no adverse effects of treatment at dietary concentrations of ≤2 ppm.

Ophthalmoscopic evaluations of the $P_1$ females and $F_1$ males and females and gross postmortem evaluations of animals of the parental generation at these same dietary concentrations (1 and 2 ppm) also demonstrated no adverse effects of treatment. Cholinesterase activities (plasma, erythrocyte and brain) were unaffected by treatment at 1 ppm. Although slight reductions in plasma and brain cholinesterase activities (19.2% and 17.4%, respectively) were seen in $F_1$ females at 2 ppm, these changes were not statistically significant from values for the controls and were not considered to be physiologically significant. Cholinesterase activities for males at 2 ppm were comparable to those of the controls (Table 9).

At 4 ppm, the following treatment-related responses were seen: tremors in several females from each parental generation; body-weight loss for days 0–21 of lactation (consistent for both litter intervals of each parental generation); reduction of plasma (74% inhibition) and brain (59% inhibition) cholinesterase activities for $F_1$ females; and reduced pup weights on days 14 and 21 of lactation (both litter intervals of the $P_1$ generation and the $F_{2a}$ litters). Pup survival indices for days 0–4 and 4–21 of lactation were lower than those for controls at 4 ppm only for the $F_{2a}$ litters. No adverse effects on body weight (other than weight loss during lactation), food consumption or mating/pregnancy/fertility indices and parturition were seen at 4 ppm. Also, ophthalmoscopic evaluations of the $F_1$ parental animals at 4 ppm revealed no treatment effects.

In the group receiving phorate at a dietary concentration of 6 ppm, the following treatment-related effects were seen: increased mortality in animals of the $F_1$ parental generation, particularly early in the pre-mating interval; tremors in the $P_1$ females and $F_1$ males and females; reduced body weights during the pre-mating periods for the $P_1$ females and $F_1$ males and females; reduced body-weight gain over the entire pre-mating interval for the $P_1$ females; reduced gestation/lactation weights (both litters of each generation); reduction in plasma (40–96% inhibition), erythrocyte (10% inhibition) and brain (40–83% inhibition) cholinesterase activities for the $F_1$ parental animals; reduced litter size at birth for the $F_{2a}$ and $F_{2b}$ litters of the $F_1$ generation; reduced litter survival indices; and reduced pup weights and pup survival indices of all litter intervals. At ophthalmoscopic evaluation, no effect of treatment at a dietary concentration of 6 ppm was seen in $P_1$ females before sacrifice, but for the $F_1$ animals, manifestations of ocular disease were noted both at initiation of the pre-mating period and at termination; ocular lesions were also seen with increased incidence among the $F_1$ animals during the weekly physical evaluations. No adverse effect of treatment at 6 ppm was evident from reproductive indices in either parental generation.

Gross postmortem evaluations of the $P_1$ and $F_1$ parental animals and selected $F_{1b}$ and $F_{2b}$ offspring after weaning revealed no adverse effect of treatment. External examination of weaned pups of treated females for each litter interval of each generation revealed no adverse effect of treatment.

Microscopic evaluation of the primary and secondary sexual organs and pituitary gland for animals of the $P_1$ and $F_1$ parental generations at 6 ppm revealed no treatment-related histomorphological alterations. Ocular lesions noted microscopically in the $F_1$ parental rats at 6 ppm were not considered to be caused by treatment, but appeared to have resulted from ocular infections acquired at an early age.

The NOAEL for parental and reproductive toxicity was 2 ppm (equal to 0.17 mg/kg bw per day for animals of the parental generations) on the basis of reduced pup growth, clinical findings, survival and inhibition of brain cholinesterase activity at 4 ppm (equal to 0.34 mg/kg bw) (Schroeder, 1991).

*(b) Developmental toxicity*

*Rats*

Groups of 25 pregnant Crl : COBS CD(SD)BR rats were given technical-grade phorate (purity, 91.7%) at a dose of 0, 0.125, 0.25 or 0.5 mg/kg bw per day by gastric intubation on days 6 to 15 of gestation, and were sacrificed on day 20 of gestation. The fetuses were removed for gross, skeletal and visceral examination. The pregnancy rate was comparable in all groups. Mortality was observed only in the group receiving phorate at a dose of 0.5 mg/kg bw per day (seven dams died). At 0.5 mg/kg bw per day, fetuses had an increased frequency of enlarged heart. Clinical signs, body weight and food consumption of dams during gestation, the number of implantation sites, the number of resorptions, the number of dead fetuses, mean live litter size, average fetal weight, sex ratio, and gross, skeletal and visceral abnormalities of fetuses were not significantly different from those of the controls. The NOAEL was 0.25 mg/kg bw per day (Beliles & Weir, 1979).

A pilot study that complied with the principles of GLP and that was certified for QA was carried out to determine the dose of technical-grade phorate to be used in a subsequent

study of developmental toxicity. Groups of eight mated female rats were given phorate (purity, 92.1%) at a dose of 0 (vehicle only—corn oil), 0.25, 0.5, 0.7 or 0.9 mg/kg bw per day on days 6–15 of gestation. The presence of spermatozoa or a copulatory plug was considered to be evidence of mating and the day on which it occurred was considered to be day 0 of gestation. Doses of ≥0.05 mg/kg bw per day were lethal; there were no surviving rats in the groups receiving phorate at a dose of 0.5, 0.7 or 0.9 mg/kg bw per day after day 12 of gestation. Overt clinical signs of toxicity preceding death included: twitches, tremors, excessive salivation, exophthalmos, urine-stained abdominal fur, ataxia, decreased motor activity, chromodacryorrhea, yellow anal/vaginal substance, chromorrhinorrhea, hyperactivity, clonic convulsion, lacrimation, soft or liquid faeces, emaciation, cold to touch, gasping and body jerks, and decreased body weight and food intake. Gross examination of rats that died revealed enlarged and/or congested adrenal glands. On the basis of these data, doses of 0, 0.1, 0.3 and 0.4 mg/kg bw per day were recommended for use in the definitive study of teratology in rats (Lochry, 1990).

A study of teratology was conducted to evaluate the developmental toxicity or the potential developmental toxicity (embryo-fetal toxicity/teratogenicity) of technical-grade phorate in Crl:CD®BR VAF/Plus® (Sprague-Dawley) rats. Groups of female rats were given phorate (purity, 92.1%) at a dose of 0 (vehicle), 0.1, 0.2, 0.3 or 0.4 mg/kg bw per day by gavage in corn oil once daily on days 6 to 15 of presumed gestation. There were 25 rats in the groups receiving phorate at a dose of 0 (vehicle) or 0.4 mg/kg bw per day, and 24 rats in each of the other treated groups. Phorate was prepared at a concentration of 0 (vehicle), 0.02, 0.04, 0.06 or 0.08 mg/ml, respectively, in a volume of 5 ml/kg, adjusted daily on the basis of the individual body weights recorded immediately before intubation. The rats were examined daily during and after dosing for clinical observations of the effects of the test substance, abortions, premature deliveries and deaths. Body weights and food consumption were recorded on day 0 of presumed gestation and daily during and after dosing. Rats that were found dead were necropsied on the day that death occurred. On day 20 of presumed gestation, the rats were sacrificed by carbon dioxide asphyxiation, and the abdomen of each rat was opened and examined for pregnancy, number and placement of implantations, early and late resorptions, live and dead fetuses and number of corpora lutea. Gravid uterine weights were recorded. Fetuses were subsequently examined for gross external, soft tissue and skeletal alterations.

Six rats receiving phorate at a dose of 0.4 mg/kg bw per day died after five to ten doses had been given (days 11–16 of presumed gestation). Of these, one rat was found dead on day 15 of presumed gestation and was not pregnant. All other rats were found dead on days 11, 15 or 16 of gestation and were pregnant. Each of these deaths were considered to be caused by administration of the test substance because of observations associated with the test substance (clinical observations, decreased body-weight gains and/or body-weight loss, decreased food consumption and necropsy lesions), and because eight out of eight rats in the groups receiving phorate at adose of 0.5, 0.7 or 0.9 mg/kg bw per day died in the pilot study.

Significantly increased numbers of rats at 0.4 mg/kg bw per day had tremors, chromodacryorrhea, urine-stained abdominal fur, decreased motor activity, chromorrhinorrhea, excess salivation, impaired righting reflex, a red substance around the nose and laboured breathing, compared with numbers in the control group. Increased numbers of rats in this group (0.4 mg/kg bw per day) had a red vaginal substance, red or tan oral substance and hunched posture, compared with numbers in the control group.

At necropsy, significantly increased numbers of rats at 0.4 mg/kg bw per day had urine-stained abdominal fur, chromodacryorrhea, a red, yellow or tan substance present around the eyes, nose and/or mouth and large adrenal glands, compared with numbers in the control group. Increased numbers of rats at 0.4 mg/kg bw per day also had a red or yellow substance present around the anal-vaginal area, as compared with numbers in the control group.

Administration of phorate at a dose of 0.4 mg/kg bw per day to the dams caused significant body weight loss, significant decreases in average maternal body-weight gains for the entire period of dosing and significantly reduced maternal body weights from day 12 of gestation until day 20 of gestation. Maternal body weight corrected for the gravid uterine weight was also significantly reduced for this group.

Significant decreases in food consumption occurred for the entire period of dosing and persisted after dosing, when compared with values for the control group. Fetal body weights were significantly decreased at 0.4 mg/kg bw per day. No other caesarean-delivery parameter was affected by administration of the test substance at doses as high as 0.4 mg/kg bw per day to the dams.

There were significant increases in the fetal and/or litter incidences of variations in skeletal ossification at 0.4 mg/kg bw per day. These variations were reversible delays in ossification of the sternum and pelvis and were expected observations that are related to the significantly decreased fetal body weights in this group. No malformations or variations revealed by gross external or soft tissue examination of the fetuses and no skeletal malformations were attributed to administration of the test substance.

The NOAEL for maternal and developmental toxicity was 0.3 mg/kg bw per day on the basis of mortality, clinical signs of toxicity, significantly decreased body weights and food consumption in the dams, and decreased fetal body weights and potentially reversible delays in skeletal ossification at 0.4 mg/kg bw per day. No fetal malformations were produced even at a (lethal) dose of 0.4 mg/kg bw per day, the highest dose tested. Therefore, phorate is not a developmental toxicant (Lochry, 1990).

*Rabbits*

A range-finding study of teratology was undertaken to provide preliminary data on the maternal toxicity, embryotoxicity and/or fetoxicity of phorate in the pregnant rabbit. Groups of five mated New Zealand white rabbits were given phorate (purity, 92.1%; dissolved in corn oil) at a dose of 0, 0.3, 0.6, 0.9, 1.2 or 1.5 mg/kg bw per day by gastric intubation on days 6–18 of gestation.

The incidences of mortality in the six groups were 0/5 (control) 1/5 (0.3 mg/kg bw per day), 1/5 (0.6 mg/kg bw per day), 1/5 (0.9 mg/kg bw per day), 2/5 (1.2 mg/kg bw per day) and 4/5 (1.5 mg/kg bw per day). Only one female at the highest dose survived to scheduled sacrifice. Food intake was generally decreased in all the treated groups, although no clear dose–response relationship was seen. Increased numbers of resorptions and postimplantation losses were observed at ≥0.6 mg/kg bw per day. Decreased mean fetal body weights and shorter crown–rump lengths were noted at 1.2 mg/kg bw per day. External examination of the fetuses showed no treatment-related malformations or alterations.

The only significant effects observed at the LOAEL of 0.3 mg/kg bw per day were a single maternal death and depressed food intake (Schroeder & Daly, 1986).

In the subsequent study of teratology in rabbits, groups of 20 mated female New Zealand white rabbits were given technical-grade phorate (purity, 92.1%; mixed with corn oil) at a dose of 0.15, 0.5, 0.9 or 1.2 mg/kg bw per day (derived from the range-finding study described above; Schroeder & Daly, 1986) by gastric intubation on days 6–18 of gestation. A control group of 20 mated females received vehicle (corn oil) only at a comparable volume as treated animals (2 ml/kg bw per day) on days 6–18 of gestation.

During gestation, females were observed twice daily for overt toxicity and morbidity or mortality. Animals were given a detailed physical examination and weighed on days 0, 6, 9, 12, 15, 18, 24 and 30 of gestation. Additionally, food consumption was recorded at several daily intervals during gestation. Animals experiencing abortion or delivering prematurely before scheduled sacrifice were killed on the day such events were seen. Surviving females were sacrificed on day 30 of gestation and given a gross postmortem examination. The ovaries and uterus were removed intact and weighed. The number of corpora lutea were recorded for each ovary and uterine implantations were identified as live, dead or resorbed fetuses. Fetuses recovered at this time were weighed, sexed (internal inspection of the gonads at visceral evaluation) and evaluated for external, visceral (microdissection procedure) and skeletal (Alizarin red S stained specimens) malformations or variations.

No treatment-related mortality was seen at a dose of 0.15 mg/kg bw per day. Mortality rates at 0.5, 0.9 and 1.2 mg/kg bw per day were 5.0% (1/20), 10.0% (2/20) and 40.0% (8/20), respectively; the mortality seen at these doses was considered to be treatment-related.

At doses of 0.15, 0.5 and 0.9 mg/kg bw per day, no adverse effects of treatment were evident from data on maternal weight, food consumption, physical observation or uterine implantation. Thus, these doses were not considered to be fetotoxic or teratogenic.

At the highest dose of 1.2 mg/kg bw per day, mean body weight on day 18 of gestation was significantly ($p < 0.05$) lower than that of the controls. During days 15–18 and 6–18 of gestation, this group experienced decreases in mean body weight and these data differed statistically ($p < 0.01$) from those for the control group, which experienced a slight gain in mean body weight over these same intervals. Food consumption at this dose was lower than that of the controls on days 15, 18 and 19 of gestation, but this was statistically significant ($p < 0.05$) only at day 18. During the physical observations, it was noted that several females receiving the highest dose had staining of the skin/fur in the ano-genital area during the treatment period; a similar observation was made at gross postmortem examination for several females that died during the treatment period. In addition to eight deaths that occurred before necropsy, one female aborted and another delivered prematurely. For the ten pregnancies evaluated at day 30 in this group receiving the highest dose, no adverse effects of treatment were evident from data on uterine implantation, fetal weights or fetal sex distribution. The only external malformation seen in the fetuses whose mothers received the highest dose was open eye, seen in all three fetuses from a single litter; these same fetuses when evaluated for skeletal malformation had curved scapulae. In this group, the female whose litter contained the fetuses with open-eye defects and curved scapulae experienced considerable toxicity as evidenced by a marked body-weight loss (778 g) during treatment and reductions in food consumption at days 15 and 18 of gestation. In the published literature, ocular malformations in rabbit fetuses have been identified as defects associated with maternal toxicity. Evaluation of the remaining 83 fetuses (nine litters) at the highest dose did not reveal an increase in malformations.

Phorate was not embryotoxic, fetotoxic or teratogenic at doses up to and including 1.2 mg/kg bw per day, a dose which produced severe maternal toxicity.

The NOAEL for maternal toxicity was 0.15 mg/kg bw per day on the basis of mortality observed at 0.5 mg/kg bw per day. The NOAEL for developmental toxicity was 1.2 mg/kg bw per day, the highest dose tested (Schroeder & Daly, 1987).

### *2.6 Special studies*

*(a) Neurotoxicity*

*(i) Single exposure*

In a study of acute neurotoxicity, which complied with the principles of GLP and that was certified for QA, groups of 20 male and 20 female Sprague-Dawley CD® rats were given a single oral dose of technical-grade phorate (purity, 91.8%) at 0.25, 0.50, or 1.0 mg/kg bw (dose volume, 5 ml/kg bw) by gastric intubation. A control group of 20 animals of each sex received vehicle only (corn oil) at the same volume as the treated animals. Analysis of the dosing suspension for the group receiving the lowest dose (0.25 mg/kg bw) indicated that the correct concentration had not been prepared and administered. Therefore an additional group of 20 rats of each sex received a single oral dose of phorate at 0.25 mg/kg bw by gavage. Because animals in this additional group were dosed at a different time than those in the groups receiving the intermediate or highest doses, an additional control group of 20 animals of each sex) was included. An additional ten females were given phorate at a dose of 1.0 mg/kg bw before the initiation of the study in order to assess survival.

Physical observations, measurements of body weight and food consumption, and neurobehavioural evaluations (motor activity and functional observational battery) were performed on all animals pretest and at selected intervals during the treatment period. Blood samples were obtained from ten animals of each sex per group at the time of peak effect (approximately 4–5 h after dosing) for measurements of plasma cholinesterase and erythrocyte acetylcholinesterase activities. Immediately after blood collection, these animals were sacrificed and brains were obtained for the measurement of acetylcholinesterase activity. Blood samples for the measurement of plasma cholinesterase and erythrocyte acetylcholinesterase activities were collected from the remaining ten animals of each sex per group at 7 and 14 days after treatment. All animals were sacrificed at 14 days after treatment and a complete macroscopic examination was performed. Brains were obtained from five animals of each sex per group immediately after blood collection for the measurement of acetylcholinesterase activity. The remaining five animals of each sex per group, designated for neuropathology, were anaesthetized with an intraperitoneal injection of sodium pentobarbital and transcardially perfused with phosphate-buffered saline followed by 1% glutaraldehyde and 4% paraformaldehyde in the same buffer for histopathological evaluation of selected tissues of the central and peripheral nervous systems.

No mortalities were observed. Clinical findings noted during daily cage-side observations and weekly physical examinations were of the type commonly found in laboratory rats and were not considered to be treatment-related.

Mean body weights and body-weight gains for males and females in the treated groups (0.25, 0.5 or 1.0 mg/kg bw) were comparable to those for the respective controls throughout the study.

There were no treatment-related effects on food consumption for males or females in any treatment group compared with the respective control groups.

There were no statistically significant changes in mean motor activity on days 1, 8 and 15 for any treated group (0.25, 0.5 or 1.0 mg/kg bw) compared with the respective control groups.

Findings typical of those expected with cholinesterase inhibitors were made during the functional observational battery evaluations on day 1 in males and females at 0.5 and 1.0 mg/kg bw. Observations consisted of miosis for two males and two females at 0.5 mg/kg bw, and two males and five females at 1.0 mg/kg bw, compared with no males and one female in the control group. Additionally, at the highest dose, tremors were noted in one female, and tremors, fasciculations, slightly impaired locomotion, and splayed/dragging hindlimbs were noted in another. Recovery was evident during the 2-week observation period after dosing as the results of functional observational battery evaluations on days 8 and 15 were normal for all treatment groups.

Statistically significant inhibition of plasma cholinesterase activity and erythrocyte and brain acetylcholinesterase activities was observed on the day of dosing for males and females at 1.0 mg/kg bw when compared with controls. Plasma cholinesterase activity was inhibited by 27.5% and 67.7%; erythrocyte acetylcholinesterase activity was inhibited by 21.4% and 65.1%; and brain acetylcholinesterase activity was inhibited by 14% and 65.2%, for males and females, respectively, when compared with controls (Table 10).

Brain acetylcholinesterase activity was statistically significantly reduced on the day of dosing for males at 0.5 mg/kg bw when compared with controls. However, based on the small magnitude of this decrease (6.1%) and the lack of a similar statistically or biologically significant decrease in brain acetylcholinesterase activity at 0.5 mg/kg bw for females (females being more sensitive to phorate-induced inhibition of cholinesterase activity), this slight decrease in brain acetylcholinesterase activity for males at 0.5 mg/kg bw was not considered to be biologically significant.

***Table 10. Inhibition of mean cholinesterase activity relative to controls in rats given a single oral dose of phorate***

| Dose (mg/kg bw per day) | Inhibition (%) of mean cholinesterase activity | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Plasma | | | Erythrocyte | | | Brain | |
| | Day | | | | | | | |
| | 1 | 8 | 15 | 1 | 8 | 15 | 1 | 15 |
| *Males* | | | | | | | | |
| 0.25 | 1.7 | 6.3 | 4.5 | –7.6[a] | 5.8 | 4.2 | 2.3 | 1.8 |
| 0.5 | –1.2[a] | –9.0[a] | –8.2[a] | 10.5 | 6.2 | –2.8[a] | 6.1* | –0.7[a] |
| 1.0 | 27.5** | 0.7 | 0.6 | 21.4** | 10.2 | 22.1 | 14.0** | 7.2 |
| *Females* | | | | | | | | |
| 0.25 | –3.5[a] | 5.7 | –2.2[a] | –17.5[a] | –1.1[a] | –16.0[a] | 0.5 | –2.3[a] |
| 0.5 | 18.7 | –0.5[a] | 0.0 | 5.1 | 17.1 | 2.3 | 3.1 | –3.1 |
| 1.0 | 67.7** | 4.0 | 7.5 | 65.1** | 27.1 | 15.1 | 65.2** | 9.3 |

From Mandella (1998)

* Significantly different from mean for controls; $p < 0.05$

** Significantly different from mean for controls; $p < 0.01$

[a] Apparent enhancement of cholinesterase activity

Recovery was evident in plasma cholinesterase activity and erythrocyte acetylcholinesterase activity by 8 days after dosing and in brain acetylcholinesterase activity by 14 days after dosing.

There were no macroscopic or microscopic findings at any dose that were related to treatment with phorate.

The NOAEL for inhibition of plasma cholinesterase and erythrocyte and brain acetylcholinesterase was 0.5 mg/kg bw on the basis of statistically significant reductions in plasma cholinesterase activity and erythrocyte and brain acetylcholinesterase activities on day 1 for males and females at 1.0 mg/kg bw, the highest dose tested. The NOAEL for neurobehavioural effects was 0.5 mg/kg bw on the basis of tremors in two females, and fasciculations, slightly impaired locomotion and splayed/dragging hindlimbs in one female at 1.0 mg/kg bw, the highest dose tested. The NOAEL for motor activity effects and neuropathology findings was 1.0 mg/kg bw, the highest dose tested. The overall NOAEL was 0.25 mg/kg bw on the basis of changes observed in the functional observational battery (miosis) in two males and two females at 0.5 mg/kg bw, the next highest dose tested (Mandella, 1998).

*(b) Repeated exposure*

In a 13-week study that complied with the principles of GLP and that was certified for QA, groups of 20 male and 20 female Sprague-Dawley CD® rats were given diets containing technical-grade phorate (purity, 91.8%) at a concentration of 0.5, 1.0, or 7.0 ppm for males and 0.5, 1.0, or 4.0 ppm for females. A control group of 20 animals of each sex received standard laboratory diet mixed with the carrier, acetone.

The dietary concentrations tested in the 13-week study were selected based on results from a pilot range-finding study of 21 days duration (Mandella, 1999b), which showed a 33% and a 87.9% decrease in erythrocyte acetylcholinesterase activity for males at 2.0 and 7.0 ppm, respectively, and a 39.4% and 100% decrease in erythrocyte acetylcholinesterase activity for females at 2.0 and 5.0 ppm, respectively. Additionally, brain acetylcholinesterase activity was decreased by 43.6% for males at 7.0 ppm and by 6.5% and 80.8% for females at 2.0 and 5.0 ppm, respectively. No effects on erythrocyte or brain acetylcholinesterase activity were noted for males and females at 1.0 ppm, the lowest concentrations tested for the respective sexes.

Mean daily intakes of phorate in the definitive study are summarized in Table 11.

***Table 11. Mean daily substance intake in rats fed diets containing phorate for 13 weeks***

| Dietary concentration (ppm) | Mean daily intake (mg/kg bw) | |
|---|---|---|
| | Males | Females |
| 0.5 | 0.037 | 0.041 |
| 1.0 | 0.074 | 0.081 |
| 4.0 | — | 0.334 |
| 7.0 | 0.538 | — |

From Mandella (1999a)

Physical observations and measurements of body weight and food consumption were performed for all animals before the start of the study and at selected intervals during the treatment period. Ophthalmoscopic examinations were conducted for all animals before the start of the study and for 10 animals of each sex per group (designated for evaluation of neurobehaviour) at termination. Motor activity and a functional observational battery (FOB) of behavioural tests were performed for 10 animals of each sex per group (designated for evaluation of neurobehaviour) before the start of the study and during weeks 4, 8 and 13 of treatment. Blood samples for the measurement of plasma cholinesterase and erythrocyte acetylcholinesterase activities were obtained from 10 animals of each sex per group (designated for evaluation of cholinesterase activity) during weeks 4, 8 and 13 of treatment and from five animals of each sex per group (designated for evaluation of neurobehaviour) at termination of treatment. At study termination, the brains from up to 15 animals of each sex per group (five animals of each sex in the group designated for evaluation of neurobehaviour and up to 10 animals of each sex in the group designated for evaluation of cholinesterase activity) were removed for measurement of acetylcholinesterase activity. The remaining five animals of each sex per group, designated for neuropathology, were anaesthetized with an intraperitoneal injection of sodium pentobarbital and transcardially perfused with phosphate-buffered saline followed by 1% glutaraldehyde and 4% paraformaldehyde in the same buffer for histopathological evaluation of selected tissues of the central and peripheral nervous systems. Complete macroscopic examinations were performed on all animals at necropsy.

Analysis of dietary mixtures confirmed that they were homogeneous. Stability analyses conducted for this study and for the range-finding study demonstrated that the test substance was stable in the diet for at least 14 days at room temperature and for at least 7 days (low concentration) or 14 days (high concentration) when stored refrigerated or frozen. Analysis of dietary mixtures during the treatment period confirmed that diets containing phorate at the appropriate concentration were administered.

There were no mortalities during the study. Clinical findings noted during daily cage-side observations and weekly physical examinations were of the type commonly found in laboratory rats and were not considered to be related to administration of the test substance. No test substance-related ocular abnormalities were noted at termination.

There were no statistically significant differences in motor activity at week 4, 8 or 13 for males or females in any treated group when compared with that of the control animals. Functional observational battery evaluations during weeks 4, 8 or 13 did not indicate any neurobehavioural effects of the test substance. There were no findings indicative of a cholinergic effect. Miosis was noted at week 4 for one of ten females at 1.0 ppm. This finding was considered to be an incidental finding and not related to treatment because the finding was not dose-related (as miosis was not noted at any interval in females at 4.0 ppm), and because miosis was not observed in the affected animal at weeks 8 or 13 after additional treatment with the test substance. A slight, but statistically significant, decrease in hindlimb grip strength was noted at week 4 for females at 1.0 ppm, relative to the control value. This decrease was not considered to be treatment-related because: the finding was not dose-related as statistically significant decreases in hindlimb grip strength were not observed at any interval in females at 4.0 ppm; a statistically significant decrease in hindlimb grip strength was only observed in one of the two trials at week 4 for females in the group at 1.0 ppm; and statistically significant decreases in hindlimb grip strength were not observed for females at 1.0 ppm at week 8 or 13 after further treatment with the test substance.

No adverse treatment-related effects on body weight, overall (weeks 1 to 13) body-weight gain or food consumption were observed.

Plasma cholinesterase activity (for animals in the groups designated for evaluation of cholinesterase activity or neurobehaviour) was statistically significantly decreased, relative to values for the respective controls, for males at 7.0 ppm and for females at 4.0 ppm. The inhibition of activity for animals in the group designated for evaluation of cholinesterase activity at weeks 4, 8 and 13 was 30.9%, 47.6% and 40.3%, respectively, for males at 7.0 ppm and 60.2%, 67.6% and 65.5%, respectively, for females at 4.0 ppm. The inhibition of plasma cholinesterase activity for animals in the group designated for evaluation of neurobehaviour at termination was 44.0% for males at 7.0 ppm and 73.6% for females at 4.0 ppm.

Erythrocyte acetylcholinesterase activity (for animals in the groups designated for evaluation of cholinesterase activity or neurobehaviour), was statistically significantly decreased, relative to values for the respective controls, for males at 7.0 ppm and for females at 4.0 ppm. The inhibition for males at 7.0 ppm was 84.4%, 91.2% and 78.4% for the animals in the group designated for evaluation of cholinesterase activity in weeks 4, 8 and 13, respectively, and 72.2% for the animals in the group designated for evaluation of neurobehaviour at termination. The inhibition for females at 4.0 ppm was 80.6%, 96.4% and 75.5% for the animals in the group designated for evaluation of cholinesterase activity at weeks 4, 8 and 13, respectively, and 82.4% for animals in the group designated for evaluation of neurobehaviour at termination. Erythrocyte acetylcholinesterase activity was decreased by 22.9% in week 4 for females at 1.0 ppm. This decrease was not statistically different from that in controls and was therefore not considered to be treatment-related. Moreover, erythrocyte acetylcholinesterase activity was decreased by only 11.1% at week 8 for females designated for evaluation of cholinesterase activity and was comparable to controls at termination for females designated for evaluation of cholinesterase activity or neurobehaviour.

Brain acetylcholinesterase activity (animals designated for evaluation of cholinesterase activity or neurobehaviour) was statistically significantly decreased at termination, relative to values for the respective controls, for males at 7.0 ppm and for females at 4.0 ppm. The inhibition of activity was 55.0% and 45.4% for males in the groups designated for evaluation of cholinesterase activity or neurobehaviour, respectively, and 66.6% and 64.7% for females, in the groups designated for evaluation of cholinesterase activity or neurobehaviour, respectively (Table 12).

There were no macroscopic findings related to administration of the test substance. No neuropathological effects were noted upon microscopic examination of tissues for males at 7.0 ppm or females at 4.0 ppm.

The NOAEL for this study was 1 ppm (equal to 0.07 mg/kg bw per day) on the basis of reduced erythrocyte and brain cholinesterase activities at the next highest dose (7 ppm in males and 4 ppm in females) (Mandella, 1999a).

*(c) Delayed neurotoxicity*

In this study of demylenation, groups of six adult white Leghorn hens were fed diets containing phorate (purity not specified) at a concentration of 0 or 40 ppm, equivalent to a dose of 5 mg/kg bw per day, for 4 weeks. Tri-ortho-tolyl phosphate at a dietary concentra-

***Table 12. Inhibition of mean cholinesterase activity relative to controls in rats fed diet containing phorate for 13 weeks***

| Dietary concentration (ppm) | Inhibition (%) of mean cholinesterase activity | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Plasma | | | | Erythrocyte | | | | Brain | |
| | Week (s) | | | | | | | | | |
| | 4 | 8 | 13 | NT | 4 | 8 | 13 | NT | 13 | NT |
| *Males* | | | | | | | | | | |
| 0.5 | –15.3° | 0.5 | –5.2° | 9.8 | 6.0 | 5.4 | 5.0 | –2.8° | 0.9 | 1.9 |
| 1 | –2.3[a] | 10.2 | 8.5 | 9.9 | 19.2 | 1.7 | 1.2 | 6.6 | 1.0 | –2.0[a] |
| 7 | 30.9* | 47.6** | 40.3** | 44.0** | 84.4** | 91.2** | 78.4** | 72.2** | 55.0** | 45.4* |
| *Females* | | | | | | | | | | |
| 0.5 | 4.3 | 7.0 | 13.8 | –24.2[a] | 12.7 | 15.8 | 2.5 | 15.2 | 0.1 | –1.7[a] |
| 1.0 | –8.0[a] | –5.4[a] | –2.3a | 14.3 | 22.9 | 11.1 | –1.4a | –4.6a | 3.4 | –2.7a |
| 4.0 | 60.2** | 67.6** | 65.5** | 73.6 | 80.6** | 96.4** | 75.5** | 82.4** | 66.6** | 64.7* |

From Mandella (1999a)

* Significantly different from mean for controls; $p < 0.05$

** Significantly different from mean for controls; $p < 0.01$

[a] Apparent enhancement of cholinesterase activity

NT: neurotoxicity group of rats, evaluated at week 13

tion of 4000 ppm was used as the positive control. Each hen was anaesthetized and immediately perfused with buffered formalin; and sections of brain, lower thoracic cord and each sciatic nerve were prepared for microscopic examination. Tri-ortho-tolyl phosphate induced loss of myelin in the nerve tissue in each hen, but phorate had no adverse effects on nerve fibres or their myelin sheaths (Morici & Levinskas, 1965).

A study of acute delayed neurotoxicity was conducted to determine potential neurotoxic effects of technical-grade phorate in mature white Leghorn hens (aged 22–23 months). In the first phase of the study, the acute oral median lethal dose ($LD_{50}$) of phorate was determined by dosing hens with phorate dissolved in corn oil. The estimated $LD_{50}$ was 14.2 mg/kg bw.

In the second phase of the study, 50 fasted hens each received a single dose of phorate at 14.2 mg/kg bw on day 0, 1 h after being given an intramuscular injection of atropine sulfate at a dose of 10 mg/kg bw. An additional 15 fasted, atropinized hens were given corn oil only and 15 hens that did not receive atropine were given tri-ortho-toyl phosphate at a dose of 500 mg/kg bw as a positive control. All surviving hens in all groups received the same doses 21 days later, except that the administered dose of atropine sulfate was changed to 30 mg/kg bw. All hens were observed daily for mortality, clinical signs and evidence for neurotoxicity. Body weights and food consumption were recorded every 3 days. All hens that died during the study and all hens that were killed at the end of the study at day 42 were subjected to gross necropsy. Those killed at the end of the study were perfused with 10% neutralized formalin; and brains, vertebral columns (with spinal cord in situ) and the entire right and left sciatic nerves were excised and fixed. Microscopic slides of neural tissue were prepared by taking a sagittal section of the entire brain (corpus striatum, cerebellum, pons), longitudinal and cross-sections of the cervical, thoracic and lumbrosacral levels of the spinal cord and both sagittal and longitudinal sections of the right and left sciatic nerves. Sections were stained with haematoxylin and eosin, and replicate sections were stained with luxol fast blue. Tissues from 10 hens in each group were examined microscopically.

Of the 50 hens that were treated with phorate, 27 died within 24 h after the first dose and 13 more died within 24 h after the second dose. Ten hens survived to the termination of the study. No hens in the vehicle control group died during the 42-day study. All 15 hens in the positive control group were killed in extremis on day 16 of the study after clinical signs of neuropathy, first observed on day 11, became progressively more severe. These signs included generalized weakness, ataxia and paralysis of the legs and wings.

Hens in the vehicle control group and hens treated with phorate had slight generalized weakness of the limbs, lasting about 2 h, shortly after each treatment of atropine sulfate; the reaction was slightly more severe hens treated with phorate and these animals also had slight to moderate ataxia for up to 2 h after treatment. However, no clinical signs of delayed neuropathy were observed in any hen in the vehicle control group or in the group receiving phorate. In comparison with those of the vehicle controls, the mean body-weight gains of hens treated with phorate were higher at days 0–21 and lower at days 21–42, while food consumption of the treated hens was lower at days 0–21 and higher at days 21–42. No gross adverse effects attributable to phorate were observed at necropsy.

Histopathological examination of the neural tissues from the hens in the positive control group revealed treatment-related lesions involving the brain, spinal cord and/or sciatic nerves in all 10 birds. Generally, mild to moderate axonal degeneration was observed in the brains of 4 out of 10 hens, in the spinal cords of 10 out of 10 hens and in the sciatic nerves of 7 out of 10 hens; Schwann cell hyperplasia was also observed in the sciatic nerves of 3 out of 10 hens. These lesions were compatible with a delayed neurotoxic response induced by tri-ortho-tolyl phosphate. Minimal to mild focal axonal degeneration of the sciatic nerves was noted in 3 out of 10 hens treated with phorate; no axonal degeneration was seen in hens in the vehicle control group. The axonal degeneration observed in hens treated with phorate was associated with interstitial infiltration of lymphoid cells, which was also observed in other treated hens and in hens in the vehicle control group. This syndrome, which was distinct from that observed in hens in the positive control group, was ascribed to lesions of a naturally occurring disease, Marek disease, and was considered not to be treatment-related.

Thus, phorate did not induce clinical or histopathological signs indicative of acute delayed neuropathology (Fletcher, 1984).

### *2.7 Studies with metabolites*

*(a) Single exposure*

In study of acute oral toxicity, which complied with the principles of GLP and was certified for QA, groups of five male and five female Sprague-Dawley rats (Crl : CD(SD)BR strain) were given phorate sulfone (purity, 99.4%; a metabolite of phorate) at a dose of 40, 20, 10, 5, 2.5, 1.75 (males and females), 1.25 and 0.625 (females only) mg/kg bw in corn oil by oral gavage. The animals were fasted overnight (approximately 18 h) before dosing. The animals were observed daily for overt signs of toxicity during the 14-day test period. Body weights were recorded on the day of dosing (day 0), day 7 and at termination (day 14). Necropsies were performed on all decedents during the study and on all survivors at the end of the 14-day observation period.

The mortality observed is summarized in Table 13.

***Table 13. Mortality in rats given a single dose of phorate sulfone by gavage***

| Dose (mg/kg bw) | Mortality (No. died/No. dosed) | | |
|---|---|---|---|
| | Males | Females | Combined |
| 40.0 | 5/5 | 5/5 | 10/10 |
| 20.0 | 5/5 | 5/5 | 10/10 |
| 10.0 | 5/5 | 5/5 | 10/10 |
| 5.0 | 5/5 | 5/5 | 10/10 |
| 2.5 | 0/5 | 5/5 | 5/10 |
| 1.75 | 0/5 | — | 0/5 |
| 1.25 | — | 3/5 | 3/5 |
| 0.625 | — | 0/5 | 0/5 |

From Fischer (1990a)

***Table 14. Mortality in rats given a single dose of phorate sulfoxide by gavage***

| Dose (mg/kg bw) | Mortality (No. died/No. dosed) | | |
|---|---|---|---|
| | Males | Females | Combined |
| 20.0 | 5/5 | 5/5 | 10/10 |
| 10.0 | 5/5 | 5/5 | 10/10 |
| 5.0 | 5/5 | 5/5 | 10/10 |
| 2.5 | 2/5 | 5/5 | 7/10 |
| 1.875 | — | 0/5 | 0/5 |
| 1.25 | 0/5 | 0/5 | 0/10 |

From Fischer (1990b)

Overt signs of toxicity were observed at all doses except 0.625 mg/kg bw. Signs of toxicity included decreased activity, salivation, tremors, chromodacryorrhea, ataxia and twitching limbs. Mortality was observed at all doses except 1.75 and 0.625 mg/kg bw. Mortality generally occurred during the first 8 h after dosing. Body-weight gains in surviving rats were generally unaffected by administration of the test substance. Gross pathological changes observed in decedents included external evidence of salivation and lacrimation, blood around the nose, congested livers and haemorrhagic lungs. There were no gross lesions observed in surviving rats which could attributed to ingestion of the test substance.

Based on the mortality data, the oral $LD_{50}$ of phorate sulfone was 3.5 mg/kg bw in male rats and 1.2 mg/kg bw in female rats (no ranges calculable). The $LD_{50}$ for both sexes combined was 2.5 mg/kg bw (no range calculable) (Fischer, 1990a).

In a study of acute oral toxicity with the phorate metabolite phorate sulfoxide, groups of five male and five female Sprague-Dawley rats (Crl : CD(SD)BR strain) were given phorate sulfoxide (purity, 91.8%) at a dose of 1.25 (both sexes), 1.875, 2.5, 5, 10 or 20 (females only) mg/kg bw in corn oil by oral gavage. The animals were fasted overnight (approximately 18 h) before dosing. The animals were observed daily for overt signs of toxicity during the 14-day test period. Body weights were recorded on the day of dosing (day 0), day 7 and at termination (day 14). Necropsies were performed on all decedents during the study and on all survivors at the end of the 14-day observation period.

The mortality observed is summarized in Table 14.

Overt signs of toxicity were observed at all doses except 1.875 mg/kg bw. Signs of toxicity included decreased activity, salivation, tremors, chromodacryorrhea and piloerection. Mortality was observed at all doses except 1.875 and 1.25 mg/kg bw. Mortality generally occurred during the first 2 h after dosing. Body-weight gains in surviving rats were generally unaffected by administration of the test substance. Gross pathological changes observed in decedents included external evidence of salivation and lacrimation, blood around the nose, chromodacryorrhea, congested kidneys and haemorrhagic lungs. There were no gross lesions observed in surviving rats which could be attributed to ingestion of the test substance.

Based on the data on mortality, the oral $LD_{50}$ of phorate sulfoxide was 2.6 mg/kg bw in male rats and 2.2 mg/kg bw in female rats (no ranges calculable). The $LD_{50}$ for both sexes combined was 2.4 mg/kg bw (no range calculable) (Fischer, 1990b).

*(b) Repeated exposure*

A 13-week study was conducted to evaluate the toxicity of phorate sulfone (purity, 92%; also containing about 6% unchanged phorate and 2% phorate sulfoxide) in groups of 30 male and 30 female Charles River CD® strain albino rats given diets containing phorate sulfone at a concentration of 0 (50 animals of each sex), 0.32, 0.80 or 2.0 ppm.

No mortality was observed in the study. There were no treatment-related changes in appearance or behaviour. Body-weight gain and increased food consumption were seen in males at 0.8 or 2 ppm, while no differences were observed in females when compared with control animals.

The mean daily intake of phorate sulfone is summarized in Table 15.

Plasma, erythrocyte and brain cholinesterase activities were measured. Plasma cholinesterase activity was reduced by 23–27% in males at 2 ppm at weeks 1, 3 and 5 and by 25–72% in females at 2 ppm at all time-points. Plasma cholinesterase activity was also inhibited (39%) in females at 0.8 ppm at weeks 1 and 3. Erythrocyte acetylcholinesterase activity was reduced by ≥20% in both males and females at most intervals. Brain acetylcholinesterase activity was inhibited (>20%) in females at 2 ppm at weeks 3, 5 and 8.

No treatment-related effects were observed in erythrocyte volume fraction, haemoglobin, total and differential leukocyte counts and kidney and liver weights. No adverse gross and microscopic alterations were recorded.

The NOAEL was 0.80 ppm, equal to 0.08 mg/kg bw per day, on the basis of inhibition of erythrocyte and brain cholinesterase activities (Hutchinson et al., 1968a).

***Table 15. Mean daily substance intake in rats fed diets containing phorate sulfone for 13 weeks***

| Dietary concentration (ppm) | Mean daily intake (mg/kg bw) | |
|---|---|---|
| | Males | Females |
| 0.32 | 0.032 | 0.043 |
| 0.80 | 0.079 | 0.107 |
| 2.0 | 0.196 | 0.259 |

From Hutchinson et al. (1968a)

***Table 16. Mean daily substance intake in rats fed diets containing phorate sulfoxide for 13 weeks***

| Dietary concentration (ppm) | Mean daily intake (mg/kg bw) | |
|---|---|---|
| | Males | Females |
| 0.32 | 0.024 | 0.028 |
| 0.80 | 0.060 | 0.068 |
| 2.0 | 0.149 | 0.172 |

From Hutchinson et al. (1968b)

In a 13-week study of toxicity, groups of 35 male and 35 female Charles River CD® strain albino rats were given diets containing phorate sulfoxide (purity, 93%; also containing about 2% unchanged phorate and 5% phorate sulfone) at a concentration of 0 (50 animals of each sex), 0.32, 0.80 or 2.0 ppm.

Two animals died during the study; a male in the control group at day 50 and a male from the group receiving phorate at 2 ppm at day 75. There were no treatment-related changes in appearance or behaviour. No significant differences in body-weight gain and food consumption were observed.

Mean daily intakes of phorate sulfoxide are summarized in Table 16.

Plasma, erythrocyte and brain cholinesterase activity was measured at weeks 1, 3, 5, 8 and 12. Plasma cholinesterase activity was significantly reduced in males at 2 ppm at week 3 (84%) and in females at 2 ppm at weeks 1 (44%), 3 (50%) and 12 (51%); plasma cholinesterase was significantly inhibited (62%) in females at 0.8 ppm at week 3. Erythrocyte acetylcholinesterase activity was significantly reduced in males at 2 ppm at weeks 1 (23%), 3 (30%) and 12 (26%) and in females at 2 ppm at weeks 3 (46%), 5 (45%), 8 (60%) and 12 (48%); Brain acetylcholinesterase activity was significantly inhibited in males at 2 ppm at weeks 3 (21%) and 8 (11%) and in females at all intervals (18–29%); brain acetylcholinesterase activity was also significantly reduced in males at 0.8 ppm at week 3 (16%).

No treatment-related effects were observed in erythrocyte volume fraction, haemoglobin, total and differential leukocyte counts or kidney and liver weights. No adverse gross and microscopic alterations were recorded.

The NOAEL was 0.80 ppm, equal to 0.060 mg/kg bw per day on the basis of brain cholinesterase inhibition (Hutchinson et al., 1968b).

## 3. Observations in humans

In a pesticide formulation plant, cases of poisoning have been reported for two workers who were engaged in the formulation of Thimet. The symptoms of intoxication were dizziness, nausea, vomiting, constricted pupils, cardiac tachycardia, excessive salivation, respiratory distress, muscle fasciculations, and pin-point pupils. After treatment with atropine and/or 2-PAM (2-pyridine-aldoximemethiodide), both men recovered. Concentrations of phorate in the air in the plant ranged from 0.07 to 14.6 µg/l. No cholinesterase measurements were reported (Young et al., 1979; WHO, 1988). In another incident, a formulator experienced neurological symptoms (not specified) after exposure to phorate while

cleaning a mixing tank. Plasma cholinesterase and erythrocyte acetylcholinesterase activities were reduced by 50% of base-line values and increased concentrations of diethyl phosphate in urine, a metabolite of phorate, were also observed (WHO, 1988). Forty male workers who were engaged in the formulation of phorate for 2 weeks developed toxic symptoms, including gastrointestinal effects, bradycardia, and neurological effects (headache, giddiness, fatigue). Dermal and ocular irritation also occurred. In 60% of the subjects, mean plasma cholinesterase activity was decreased by 55% at the end of the first week and by 71% at the end of the second week compared with pre-exposure activity. Within 10 days after cessation of exposure, cholinesterase activity had recovered to 70% of pre-exposure levels (Kashyap et al., 1984).

## Comments

In rats treated orally with radiolabelled phorate, 77% of the administered dose was recovered in the urine within 24 h after dosing. Faecal excretion accounted for approximately 12% of the administered dose. Over the total duration of the study (192 h), essentially the entire administered dose was eliminated by excretion.

Phorate was highly toxic when administered orally, dermally or by inhalation. The oral $LD_{50}$s for rats were 3.7 mg/kg bw in males and 1.4 mg/kg bw in females. The dermal $LD_{50}$s for rats were 9.3 mg/kg bw in males and 3.9 mg/kg bw in females. The $LC_{50}$s for rats after an exposure of 1 h were 0.06 and 0.011 mg/l of air in males and females respectively. Studies of dermal and eye irritation and of dermal sensitization were not performed owing to the high acute toxicity of phorate by dermal contact.

The toxicological effects of phorate are associated with inhibition of acetylcholinesterase activity. Inhibition of acetylcholinesterase activity and clinical signs occurred at similar doses in rats, rabbits and dogs, while mice appeared to be somewhat less sensitive. The NOAELs for toxicologically significant inhibition of brain acetylcholinesterase activity were 0.05–0.07 mg/kg bw per day in 13-week and 2-year studies in rats and in 1-year studies in dogs. The NOAELs for clinical signs were generally higher. The Meeting noted that the dose–response curve for acetylcholinesterase inhibition is steep.

In an 18-month study in mice and in a 24-month study in rats, phorate did not increase the incidence of tumours or cause any non-neoplastic effects other than clinical signs secondary to inhibition of acetylcholinesterase activity.

Phorate was tested for genotoxicity in vitro and in vivo in an adequate battery of assays. In view of the lack of genotoxicity in vitro and in vivo, and on the basis of the results of studies of carcinogenicity in rodents, the Meeting concluded that phorate is not likely to pose a carcinogenic risk to humans.

In a multigeneration study of reproductive toxicity in mice, the NOAEL was 1.5 ppm (equal to 0.30 mg/kg bw per day) on the basis of slightly reduced lactation indices in four out of the six litters at 3 ppm (equal to 0.60 mg/kg bw per day).

In a two-generation study of reproductive toxicity in rats, phorate showed effects on pup growth and mortality at maternally toxic doses. The NOAEL was 2 ppm (equal to 0.17 mg/kg bw per day) on the basis of decreased brain acetylcholinesterase activity,

decreased parental and pup body weights and decreased pup survival at 4 ppm (equal to 0.35 mg/kg bw per day).

In a study of developmental toxicity in rats, the NOAELs for maternal and developmental toxicity with phorate were 0.3 mg/kg bw per day on the basis of mortality, cholinergic clinical signs of toxicity, significantly decreased body weights and food consumption in the dams, decreased fetal body weights and delays in skeletal ossification at 0.4 mg/kg bw per day. No fetal malformations were produced, even at the lethal dose of 0.4 mg/kg bw per day, the highest dose tested. Therefore, the Meeting concluded that phorate is not a teratogen in rats.

Phorate was not embryotoxic, fetotoxic or teratogenic in rabbits at doses of up to and including 1.2 mg/kg bw per day, a dose that produced severe maternal toxicity. The NOAEL for maternal toxicity with phorate was 0.15 mg/kg bw per day on the basis of mortality observed at 0.5 mg/kg bw per day. The NOAEL for developmental toxicity was 1.2 mg/kg bw per day, the highest dose tested.

The Meeting concluded that the existing database on phorate was adequate to characterize the potential hazards to fetuses, infants and children.

In a study of acute neurotoxicity in rats treated by gavage, phorate at a dose of 1 mg/kg bw caused miosis in 2 out of 20 males and 5 out of 20 females, tremors in 2 out of 20 females, fasciculations, slightly impaired locomotion and splayed or dragging hindlimbs in one female, and significant inhibition of brain and erythrocyte acetylcholinesterase activity in females (65%), but not in males (14–21%). No histopathological signs were observed. At 0.5 mg/kg bw, miosis was observed in 2 out of 20 males and 2 out of 20 females. Although miosis was observed in a small number of animals (and in 1 out of 20 controls) in the absence of inhibition of erythrocyte and brain acetylcholinesterase activity, it could not be dismissed as a compound-related effect. The NOAEL was 0.25 mg/kg bw on the basis of miosis.

Phorate did not cause acute delayed neurotoxicity in hens. Although measurements of neuropathy target esterase were not carried out, the Meeting noted that the dose used (approximately equal to the $LD_{50}$) was sufficiently high to indicate that dietary exposure to phorate would not cause delayed polyneuropathy.

The toxicity of the mammalian and plant metabolites of phorate, phorate sulfone and phorate sulfoxide, was similar to that of the parent compound. In rats, the oral $LD_{50}$s for these metabolites were 1.2–3.5 and 2.2–2.6 mg/kg bw, respectively. The NOAELs for inhibition of brain acetylcholinesterase activity were 0.80 ppm (equal to 0.08 and 0.06 mg/kg bw per day) for phorate sulfone and sulfoxide respectively in 90-day studies in rats.

Several cases of occupational and non-occupational poisoning in humans have been reported. The subjects showed typical cholinergic symptoms, including gastrointestinal effects, bradycardia and neurological effects (headache, giddiness, fatigue). Dermal and ocular irritation were also observed.

## Toxicological evaluation

An ADI of 0–0.0007 mg/kg bw was established on the basis of a overall NOAEL of 0.07 mg/kg bw per day for inhibition of brain acetylcholinesterase activity in rats and dogs, and a safety factor of 100. This ADI includes the phorate metabolites, phorate sulfone and phorate sulfoxide.

An acute reference dose (ARfD) of 0.003 mg/kg bw was also established based on the NOAEL of 0.25 mg/kg bw for miosis in the study with single doses in rats. Although inhibition of acetylcholinesterase activity is a $C_{max}$-dependent phenomenon, a safety factor of 100 was used in view of the steep dose–response curve and the slow recovery of brain acetylcholinesterase activity because of irreversibility of its inhibition. This ARfD includes phorate sulfone and phorate sulfoxide.

***Levels relevant to risk assessment***

| Species | Study | Effect | NOAEL | LOAEL |
|---|---|---|---|---|
| Mouse | 18-month study of toxicity and carcinogenicity[a] | Toxicity | 3 ppm, equivalent to 0.45 mg/kg bw per day | 6 ppm, equivalent to 0.90 mg/kg bw per day |
| | | Carcinogenicity | 6 ppm, equal to 0.90 mg/kg bw per day[d] | — |
| | Multigeneration study of reproductive toxicity[a] | Parental and offspring toxicity | 1.5 ppm, equal to 0.30 mg/kg bw per day | 3 ppm, equal to 0.60 mg/kg bw per day |
| Rat | 2-year study of toxicity and carcinogenicity[a] | Toxicity | 1 ppm, equal to 0.05 mg/kg bw per day | 3 ppm, equal to 0.16 mg/kg bw per day |
| | | Carcinogenicity | 6 ppm, equal to 0.32 mg/kg bw per day[c,d] | — |
| | Multigeneration reproductive toxicity[a] | Parental and offspring toxicity | 2 ppm, equal to 0.17 mg/kg bw per day | 4 ppm, equal to 0.35 mg/kg bw per day |
| | Developmental toxicity[a] | Embryo- and fetotoxicity and maternal toxicity | 0.3 mg/kg bw per day | 0.40 mg/kg bw per day |
| | Single-dose study[c] | Miosis | 0.25 mg/kg bw | 0.50 mg/kg bw per day |
| | 13-week study of neurotoxicity[a] | Neurotoxicity | 0.07 mg/kg bw per day | 0.3 mg/kg bw per day |
| Rabbit | Developmental toxicity[a] | Maternal toxicity | 0.15 mg/kg bw per day | 0.50 mg/kg bw per day |
| | | Embryo- and fetotoxicity[a] | 1.2 mg/kg bw per day[d] | — |
| Dog | 1-year study of toxicity[b] | Toxicity | 0.05 mg/kg bw per day | 0.25 mg/kg bw per day |

[a] Diet
[b] Capsules
[c] Gavage
[d] Highest dose tested

*Estimate of acceptable daily intake for humans*

0–0.0007 mg/kg bw

*Estimate of acute reference dose*

0.003 mg/kg bw

*Studies that would provide information useful for the continued evaluation of the compound*

Further observation in humans

***Summary of critical end-points for phorate***

| | |
|---|---|
| *Absorption, distribution, excretion and metabolism in animals* | |
| Rate and extent of oral absorption | Rapid, approximately 90% within 24 h |
| Dermal absorption | Extensive based on acute toxicity |
| Distribution | Rapid and extensive |
| Potential for accumulation | None |
| Rate and extent of excretion | 89% within 24 h; urinary excretion predominated (77%); faecal excretion (12%) |
| Metabolism in animals | Major pathway: cleavage of phosphorus–sulfur bond, methylation of the liberated thiol group and oxidation of the resulting divalent moiety to the sulfoxide and sulfone |
| Toxicologically significant compounds (plants, animals and the environment) | Parent, phorate sulfoxide and phorate sulfone |
| *Acute toxicity* | |
| Rat, $LD_{50}$, oral | 3.7 mg/kg bw in males, 1.4 mg/kg bw in females |
| Rat, $LD_{50}$, dermal | 9.3 mg/kg bw in males, 3.9 mg/kg bw in females |
| Rat, $LC_{50}$, inhalation | 0.06 mg/l of air in males (1 h), 0.011 mg/l of air (1 h) in females |
| Rabbit, dermal irritation | Highly toxic by skin contact—could not be tested |
| Rabbit, ocular irritation | Highly toxic by eye contact—could not be tested |
| Dermal sensitization | Highly toxic by skin contact—could not be tested |
| *Short-term studies of toxicity* | |
| Target/critical effect | Brain and erythrocyte acetylcholinesterase activity, and miosis (rats) |
| Lowest relevant oral NOAEL | 0.07 mg/kg bw per day |
| Lowest relevant dermal NOAEL | No data |
| Lowest relevant inhalation NOAEC | No data |
| *Genotoxicity* | Negative results in vivo and in vitro |
| *Long-term studies of toxicity and carcinogenicity* | |
| Target/critical effect | Inhibition of erythrocyte and brain cholinesterase activity |
| Lowest relevant NOAEL | 0.07 mg/kg per day (rat) |
| Carcinogenicity | Not carcinogenic in mice and rats |
| *Reproductive toxicity* | |
| Reproduction target/critical effect | Reduced pup growth at maternally toxic dose |
| Lowest relevant reproductive NOAEL | 2 ppm, equivalent to 0.17 mg/kg bw per day |
| Developmental target/critical effect | Decreased pup weights and delayed ossification at maternally toxic doses (rats) |
| Lowest relevant developmental NOAEL | 0.3 mg/kg bw per day (rats) |
| *Neurotoxicity/delayed neurotoxicity* | |
| Single dose study of neurotoxicity | |
| Target/critical effect | Signs consistent with acetylcholinesterase inhibition; no neuropathological effects |
| Relevant NOAEL | 0.25 mg/kg bw |
| Delayed neuropathy | No delayed neurotoxicity in hens |
| *Medical data* | Findings consistent with inhibition of acetylcholinesterase activity; no record of permanent sequelae |

| Summary | Value | Study | Safety factor |
|---|---|---|---|
| ADI | 0–0.0007 mg/kg bw | Rats and dogs, short- and long-term studies, inhibition of brain acetylcholinesterase activity | 100 |
| ARfD | 0–0.003 mg/kg bw | Rats, single-dose study, miosis | 100 |

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