PROFENOFOS

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Explanation

Profenofos is the International Organization for Standardization (ISO) approved name for (*RS*)-*O*-4-bromo-2-chlorophenyl *O*-ethyl S-propyl phosphorothioate (International Union of Pure and Applied Chemistry, IUPAC). The Chemical Abstracts Service (CAS) chemical name for profenofos is *O*-(4-bromo-2-chlorophenyl) *O*-ethyl S-propyl phosphorothioate (CAS No. 41198-08-7). It is a broad-spectrum organophosphorus insecticide that is used to control insect pests in cotton, maize, sugar beet, soya bean, potato, vegetables and other crops. Its mode of action is by inhibition of acetylcholinesterase activity. Profenofos is a racemic mixture of the two optical isomers at the chiral phosphorus atom. The S (−) isomer is a markedly more potent inhibitor of acetylcholinesterase in vitro than the R (+) isomer.

Profenofos was previously evaluated by JMPR in 1990 (Annex 5, reference *171*) and an acceptable daily intake (ADI) of 0–0.01 mg/kg bw per day was established. The ADI was based on the no-observed-adverse-effect level (NOAEL) of 20 ppm, equal to 1.0 mg/kg bw per day, the highest dose tested, in a three-generation study of reproduction in rats.

Profenofos was re-evaluated by the present meeting within the periodic review programme of the Codex Committee on Pesticide Residues (CCPR). All pivotal studies with profenofos were certified as complying with good laboratory practice (GLP).

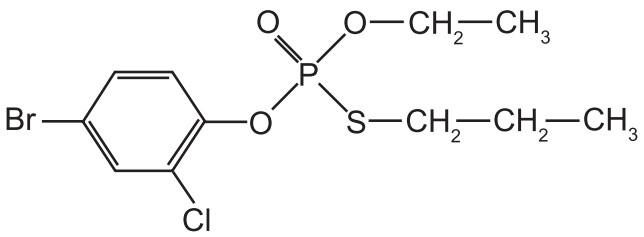
Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

RAI rats (four males and three females) received a single oral dose (gavage) of ring-labelled [^{14}C]profenofos (specific activity, 9.79 µCi/mg (362.23 kBq/mg) at approximately 4.8 mg/kg bw in ethanol : polyethylene glycol 200 : water (25 : 25 : 50). Urine and faeces were collected for 6 days after dosing and selected tissues were removed at necropsy. Essentially all the administered radioactivity was eliminated in the urine (males, 81.8%; and females, 96.4%) and faeces (males, 15.7%; and females, 2.5%) within 6 days. Most of the urinary and faecal excretion occurred within the first 24 h after dosing. The elimination half-life for excretion was less than 8 h for both sexes. Only minor amounts of $^{14}CO_2$ were eliminated in expired air (males, 0.08%; and females, 0.07%). When the rats were killed 6 days after dosing, detectable amounts of radioactivity were present only in the liver (males, 0.013 µg equivalent/g; and females, 0.023 µg equivalent/g) and kidneys (males, 0.007 µg equivalent/g; and females, 0.008 µg equivalent/g). The concentration of radioactivity in blood, fat, muscle, testis, ovary and brain was below the limit of quantification (for blood) or detection (Ifflaender & Mücke, 1974).

Figure 1. Chemical structure of profenofos



Similar results were obtained in a more recent study in which three groups of five male and five female Crl:CD®BR rats were given [^{14}C]profenofos (purity, 97.1–98.1%) orally by gavage as a single dose at 1 mg/kg bw or 100 mg/kg bw and another group was pre-treated with 14 consecutive daily oral doses of non-radiolabelled compound at 1 mg/kg bw followed by a single radiolabelled dose at 1 mg/kg bw on day 15. Treated rats were killed 7 days after administration of the radiolabelled dose.

No significant sex-related differences were observed in the elimination and/or distribution of radioactivity. Total radioactivity eliminated via the urine and faeces exceeded 99% of the administered dose for all groups. Most of the administered radioactivity (> 97%) was eliminated via the urine. Elimination was rapid with an average of 95% and 93% of the total radioactivity being excreted in the urine within the first 24 h for the groups at the lower dose and repeated dose, respectively. For the group at the higher dose, 97% of the dose was eliminated in the urine within 48 h. Less than 4% of the radioactivity was excreted in the faeces for all groups. Less than 0.2% of the administered dose was detected in the volatile and CO_2 traps combined. Less than 0.1% of the administered dose was recovered in tissues. At the lower dose, the residues in tissues were less than 0.002 µg equivalent/g, with the exception of the liver, which contained up to 0.02 µg equivalent/g. At the higher dose, residues ranged from not detectable in most tissues to 0.18 µg equivalent/g in the liver (Kennedy & Swain, 1992).

Male and female Harlan SD albino rats received single dermal applications of ring-labelled [^{14}C]profenofos at a dose of 0.5 mg/kg bw (specific activity, 9.34 µCi/mg (345.58 kBq/mg) and 10 mg/kg bw (specific activity, 2.6 µCi/mg (96.2 kBq/mg) in a 72-h balance study. Over the 72-h absorption period, the total recoveries of radiolabelled carbon averaged 92–95% of each applied dose in each sex (urine, 80–86%; faeces,, 2.2–3.9%; tissues, 0.09–1.8%; blood, 0.06% or less; treated skin, 3% or less; and cage-washings, 5% or less). Excretion in expired carbon dioxide (CO_2) was negligible (less than 0.02%, as determined from a preliminary study using the highest dermal dose). The dermal absorption was approximately 85% of the applied dose in 72 h, which indicated that [^{14}C]profenofos was absorbed at nearly the same rate for males and females regardless of the dose; t1/2 absorption values were 17.9 h and 15.0 h after treatment with the lower dose in males and females, respectively, and 16.7 h and 14.1 h after treatment with the higher dose in males and females, respectively. The calculated 50% excretion rates (urine was the major route of excretion) occurred 18.1 h and 17.4 h after treatment with the lower dose in males and females, respectively, and 23.2 h and 18.7 h after treatment with the higher dose in males and females, respectively. Fifty percent of [^{14}C]profenofos was excreted shortly after 50% had been absorbed, indicating that profenofos and its metabolites were rapidly excreted, i.e. there was no lag-time between absorption and excretion. Levels of radioactivity in selected tissues, organs (liver, kidney) and blood reached a maximum after 2 h, had reached a plateau by 8 h, and declined rapidly by 72 h after application. The total recovery after 72 h averaged 92–95% of the applied dose in males and females, 80–86% in urine and 2–4% in faeces (Williams et al., 1984).

1.2 Biotransformation

Urine collected in the 0–24 h after giving RAI rats a single oral dose of profenofos at approximately 4.8 mg/kg bw was analysed by thin-layer chromatography (TLC). Four metabolites were detected and no unchanged profenofos was present, indicating complete degradation of profenofos. The only metabolite identified by TLC was the phosphorous ester cleavage product, 4-bromo-2-chlorophenol. This metabolite did not appear in freshly-obtained urine, indicating that other labile metabolites are cleaved to this phenol (Ifflaender & Mücke, 1974).

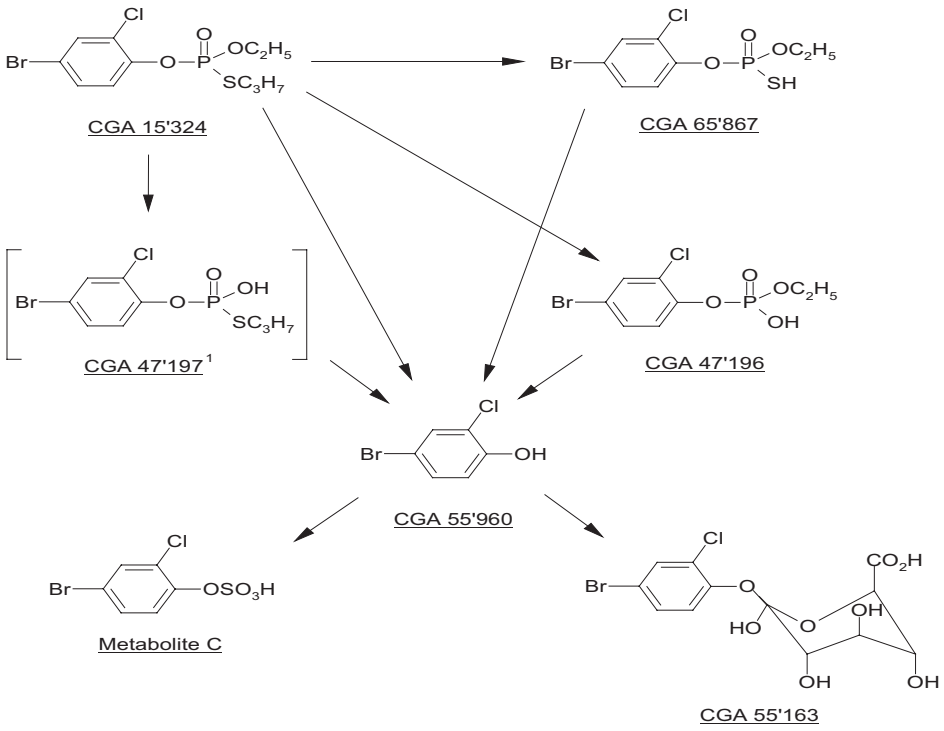
The metabolism of ring-labelled [^{14}C]profenofos (specific activity, 26.2 µCi/mg (969 kBq/mg) was investigated using the urine and faeces collected over a 2-day period after giving eleven male RAI rats a single oral dose at 28.5 mg/kg bw; 90.4% and 3.6% of the administered dose was excreted in urine and faeces within 24 h. The major metabolites detected were CGA 65867 (7%), CGA 47196 (23%), CGA 55163 (26%) and metabolite C (34%). CGA 47197 was present as a minor metabolite (< 0.5%). Neither unchanged profenofos nor its corresponding phenol CGA 55960 was detected in freshly-obtained urine. The faeces contained small amounts of parent profenofos (2%), and CGA 55960 (1%). Additional metabolites were present in minute amounts (0.2% or less) but were not identified (Ifflaender & Mücke, 1976).

A small amount (approximately 7%) of CGA 55960 was found in the urine of male rats (Tif:RAI-f strain) dosed orally with single doses of ring-labelled [^{14}C]profenofos at 0.19 or 1.80 mg/kg bw. The main urinary metabolites identified in this study were similar to those reported previously by Mücke and colleagues (Mücke, 1986).

The pattern of metabolites was investigated in urine and faeces of groups of five male and five female Crl:CD®BR rats given [^{14}C]profenofos as a single dose at 1 or 100 mg/kg bw, or [^{14}C] profenofos as a single dose at 1 mg/kg bw after 14 consecutive daily oral doses of non-radiolabelled profenofos at 1 mg/kg bw. The metabolites identified in the urine were metabolite C – the sulfated phenol (37–48%), CGA 55163 (22–27%), CGA 47196 (14–25%), CGA 65867 (5–16%), and CGA 55960 (0.1–1.7%). Major faecal components consisted of small amounts of profenofos (< 0.1–1.3%) and CGA 55960 (0.7–1.9%) for all groups. The minor amounts of faecal radioactivity (combined, 0.2–1.3%) contained partially or all of the urinary metabolites (Kennedy & Swain, 1992).

An overview of the proposed metabolic pathways of profenofos in rats is given in Figure 2.

Figure 2. Proposed metabolic pathways of profenofos in the rat



1.3 Effects on enzymes and other biochemical parameters

Profenofos is stereospecifically converted to a more potent inhibitor of acetylcholinesterase by oxidation of the sulfur on the phosphorus by a liver microsomal mixed-function oxidase system in the mouse. The net effect of this was that the chiral S (−) isomer was a 34 times more potent inhibitor of acetylcholinesterase in vitro, while the less toxic R (+) isomer was metabolically deactivated by a factor of 2, via esteratic cleavage. Prior treatment with inhibitors of mixed function oxidase markedly decreased the activation and also protected against inhibition of brain acetylcholinesterase activity and cholinergic symptoms resulting from administration of S-profenofos in chicks (Wing et al., 1983). Acetylcholinesterase activity inhibited by the S-isomer did not reactivate, probably as a result of rapid ageing (Glickman et al., 1984).

Table 1. Results of studies of acute toxicity with profenofos

Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	LC_{50} (mg/l air)	Reference
Rat	Tif: RAI (SPF)	Males and females	Oral	358 (range, 318–403)	—	Bathe (1974a)
Rat	Tif: RAIf (SPF)	Males and females	Oral	502 (range, 391–727)	—	Kobel (1983)
Rat	HSD:(SD)	Males and females	Oral	Males: 492 (range, 363–666) Females: 809 (range, 600–1090) Males and females: 630	—	Kuhn (1990a)
Rat	Sprague-Dawley	Females	Oral	1178 (range, 630–2000	—	Merkel (2004a)
Rat	Sprague-Dawley	Females	Oral	621 (range, 350–1100)	—	Durando (2005a)
Mouse	Tif: MAG (SPF)	Males and females	Oral	298 (range, 268–332)	—	Bathe (1974b)
Rabbit	Russian	Males and females	Oral	700	—	Sachsse (1974a)
Rat	Tif: RAI (SPF)	Males and females	Dermal	3300	—	Bathe (1974c)
Rat	Sprague-Dawley	Males and females	Dermal	> 2000	—	Merkel (2004b)
Rat	Sprague-Dawley	Males and females	Dermal	> 2000	—	Durando (2005b)
Rabbit	Mixed breed	Males and females	Dermal	472 (range, 304–733)	—	Sachsse (1974b)
Rabbit	New Zealand White	Males and females	Dermal	Males: 111 (range, 84.5–145) Females: 155 (range, 124–193) Males and females: 131 (range, 110–156)	—	Cannelongo (1982a)
Rabbit	New Zealand White	Males and females	Dermal	2560 (range, 2190–2990)	—	Kuhn (1989)
Rat	Sprague-Dawley Crl:CD (SD) BR	Males and females	Inhalation (4-h, whole-body)	—	3.36	Horath (1982)
Rat	Wistar-derived Alpk:APfSD	Males and females	Inhalation (4-h, nose-only)	—	> 2.2	Rattray (2004)
Rat	Sprague-Dawley	Males and females	Inhalation (4-h, nose-only)	—	> 2.03	Durando (2005c)

2. Toxicological studies

2.1 Acute toxicity

Profenofos has a moderate toxic potential in laboratory animals via the oral route of exposure with median lethal dose (LD_{50}) values ranging from 298 to 1178 mg/kg bw. It is practically non-toxic when applied to the abraded or intact skin in rats and rabbits (LD_{50}, > 2000 mg/kg bw and 3000 mg/kg bw, respectively). However if the compound is massaged into the shaved skin and kept under occlusive dressing it can be moderately toxic to rabbits (LD_{50}, ≥ 131 mg/kg bw). Whole body or nose-only inhalation exposure for 4 h is not lethal to rats (LC_{50}, > 2.0 mg/l). In laboratory animals, single oral or dermal exposure and exposure by inhalation for 4 h causes clinical signs of toxicity that are characteristic of organophosphorus compounds. These include piloerection, salivation, lacrimation, hunched posture, sedation, prostration, tremors and ataxia. The results of studies of acute oral toxicity suggested that the clinical signs characteristic of exposure to organophosphorus compounds were observed at doses greater than 100 mg/kg bw.

(a) Oral administration

Rats

Five studies were available for review. In the first study, groups of five male and five female fasted adult Tif:RAI rats were given profenofos (purity unknown) as a single dose at 215, 278, 359 or 600 mg/kg bw by gavage in 2% aqueous carboxymethylcellulose. Treated rats were examined by gross necropsy at the end of a 14-day observation period. The study was not conducted in accordance with GLP regulations.

Within 48 h, 100% mortality was observed at 600 mg/kg bw, while only one male and two females died at 359 mg/kg bw. Clinical signs, seen in all dosed groups within 2 h of treatment, but more accentuated with increased doses, were sedation, dyspnoea, exophthalmos, ruffled fur, trismus tonic-clonic muscle spasms and hunched body posture. No other details of clinical signs (incidence or duration of observation) were provided in the study report. Surviving rats recovered within 6–7 days. No effects on body weights were observed within 14 days. Necropsy of rats that were killed or that died during the study did not show any substance-related gross changes to organs. The calculated oral LD_{50} (with 95% confidence interval) for males and females combined was 358 (95% CI, 318–403) mg/kg bw (Bathe, 1974a).

In a second study, groups of five male and five female fasted Tif:RAIf rats were given profenofos (purity, 88.5%) as a single dose at 100, 200, 450 or 800 mg/kg bw by gavage in 0.5% aqueous carboxymethylcellulose. Rats were observed for a 14 days. This study was not conducted in accordance with GLP regulations.

All rats of the group at 800 mg/kg bw and one male and one female from the group at 450 mg/kg bw died within 3 days, while surviving rats recovered from all clinical signs within 13 days. Clinical signs seen within 1 h of dosing in all groups were similar to those observed in the previous study. At 100 and 200 mg/kg bw, the clinical signs were non-specific. Clinical signs in rats in the control group were not reported. Rats in the groups at 450 and 800 mg/kg bw exhibited tremors, sedation and loss of postural tone. The surviving rats gained weight during the study period. The oral LD_{50} in male and female rats was calculated to be 491 (95% CI, 325–1016) and for males and females combined it was 502 (95% CI, 391–727) mg/kg bw (Kobel, 1983).

In the third study, groups of five male and five female fasted Harlan Sprague-Dawley rats were given undiluted profenofos technical (purity unknown) as a single dose at 200, 400 or 1000 mg/kg

bw by gavage. An additional five females received profenofos at a dose of 1500 mg/kg bw. After a 14-day observation period, gross necropsy was performed. This study was conducted in accordance with GLP regulations.

Most rats in the groups at 1500 (five out of five) and 1000 mg/kg bw (nine out of ten) died within the first 3 days after treatment. At 200 and 400 mg/kg bw, piloerection was seen in all rats within 0.5 h after dosing. Diarrhoea was seen in one male at 400 mg/kg bw, 6 h after dosing. No other clinical signs were observed during a 14-day observation period in the groups at 200 and 400 mg/kg bw. After 0.5 h from dosing, prominent clinical signs seen in the groups that received 1000 and 1500 mg/kg bw included piloerection, decreased activity, aggression, ataxia, tremors, diarrhoea, exophthalmus, gasping, lacrimation, nasal discharge, polyuria, prolapsed penis and salivation. These symptoms disappeared within 6 days in surviving rats. Body weights were unaffected by the treatment. At gross necropsy, discoloration of the gastrointestinal contents and gastrointestinal tract distended with gas were further findings. The acute oral LD50 values in rats were 492 (95% CI, 363–666) for males and 809 (95% CI, 600–1090) mg/kg bw for females. For males and females combined, the oral LD_{50} was calculated to be 630 mg/kg bw (95% CI undefined) (Kuhn, 1990a).

Two studies had been conducted in fasted rats using the "up-and-down" procedure.

In the first study, there were no signs of toxicity in one female Sprague-Dawley rat that received profenofos (purity, 92.1%) as a single dose at 199 mg/kg bw. Three female rats were subsequently given profenofos as single dose at 630 or 2000 mg/kg bw and observed for 14 days. The study was conducted in accordance with GLP regulations.

All rats at 2000 mg/kg bw died with 1 day of dosing. Rats at 2000 and 630 mg/kg bw exhibited clinical signs of toxicity that consisted of ocular discharge, facial staining, hypoactivity, hunched posture, anogenital staining and reduced faecal volume. There were no mortalities and no clinical signs of toxicity 10 days after treatment. All rats gained weight over the 14-day experimental period but at 630 mg/kg bw one rats lost weight between days 0 and 7. Gross necropsy of rats that died in the group at 2000 mg/kg bw revealed discoloration of the intestines. The LD_{50} for males and females combined was 1178 (95% CI, 630–2000) mg/kg bw (Merkel, 2004a).

In the second study using the "up-and-down" procedure, three female Sprague-Dawley rats, fasted overnight, were given profenofos (purity, 89.0%) as a single dose at 350 or 1100 mg/kg bw . Treated rats were examined by gross necropsy at the end of a 14-day observation period. This study was conducted in accordance with GLP regulations. All rats that received 1100 mg/kg bw died within 2 days of dosing. Ocular discharge, hypoactivity, abnormal posture, piloerection, anogenital staining and reduced faecal volume were noted before death and necropsy revealed discoloration of the intestines. There were no signs of toxicity amongst the rats at 350 mg/kg bw; all rats gained weight The LD_{50} for female rats was calculated to be 621 (95% CI, 350–1100) mg/kg bw (Durando, 2005a).

Mice

Groups of five male and five female fasted Tif:MAG mice were given profenofos (purity unknown) as a single dose at 215, 278, 317 or 464 mg/kg bw by gavage formulated in 2% aqueous carboxymethyl cellulose. Mice were observed for 14-days. The study was not conducted in accordance with GLP regulations. At 215, 278, 317 and 464 mg/kg bw, 1 out of 10, 3 out of 10, 6 out of 10 and 10 out of 10 mice died within 48 h. Surviving rats showed sedation, dyspnoea, and exophthalmus, curved position and ruffled fur within 2 h of dosing. At 464 mg/kg bw, tonic-clonic muscle spasms were observed. No other details of clinical signs (incidence and duration of observation) were provided in the study report. Surviving rats recovered within 4–12 days. At necropsy, no gross changes in organs were seen. The oral LD50 for male and female mice was calculated to be 298 (95% CI, 268–332) mg/kg bw (Bathe, 1974b).

Rabbits

Groups of two male and two female Russian-breed rabbits were given profenofos (purity unknown) as a single dose at 100, 600, 1000 or 2150 mg/kg bw in 2% aqueous carboxymethyl cellulose by oral intubation. Rabbits were observed for only 7 days. The study was not conducted in accordance with GLP regulations. Within 24 h, all rabbits at 2150 mg/kg bw and one male and two females at 600 mg/kg bw had died; only one male at 600 mg/kg bw died at 48 h after dosing. None of the rabbits in the group at the lowest dose (100 mg/kg bw) died or showed any signs of toxicity. In other groups, within 2–3 h after dosing, rabbits showed salivation, ataxia, exophthalmus, lateral position and sedation, these being more accentuated in groups at higher doses. No other details of clinical signs (incidence and observation duration) were provided in the study report. The surviving rabbits recovered within 4–12 days. Necropsy of these animals revealed no gross changes to organs; congested organs were noted in rabbits that died during the study. The oral LD50 for male and female rabbits was calculated to be approximately 700 mg/kg bw (95% confidence interval undefined) (Sachsse, 1974a).

(b) Dermal administration

Rats

Groups of three male and three female Tif:RAI rats received a single application of profenofos (purity unknown) at a dose of 2150, 2780 or 3170 mg/kg bw. Profenofos was applied as a concentrate under occlusive conditions (with a plaster and aluminium foil) to the shaved back of the rat. After 24 h, the plaster and aluminium foil were peeled off and the skin was washed with warm water. Rats were observed for clinical signs and mortality for 14 days. This study was not conducted in accordance with GLP regulations. Two male rats at the highest dose died within 7 days after application of the substance. There were no deaths in other groups. Within 24 h after treatment, rats at all doses showed sedation, dyspnoea, loss of postural tone, and ruffled fur. In the group at the highest dose, slight erythema was observed. Surviving rats recovered within 5–8 days. At necropsy, no substance-related gross changes to organs were seen. The dermal LD_{50} was calculated to be approximately 3300 mg/kg bw in males and females (Bathe, 1974c).

In two more recent studies, groups of five male and five female Sprague-Dawley rats were given profenofos (two batches; purity, 92.1%; and purity, 89.0%) at a dose of 2000 mg/kg bw applied once to the skin for 24 h. These studies were conducted in accordance with GLP regulations. In the first study, there were no signs of toxicity or dermal irritation and no treatment-related gross abnormalities were noted in any rat at necropsy 14 days after treatment (Merkel, 2004b). All rats gained weight during the observation period. Similar results were obtained in the second study except that dermal irritation (erythema and oedema) was noted in four of the female rats 1 and 2 days after treatment. Neither the severity of irritation nor the scoring method was given in the study report. In the second study, the lot number and purity of profenofos was different to that used in the first study. The dermal LD_{50} was > 2000 mg/kg bw in male and female rats (Durando, 2005b).

Rabbits

Dermal toxicity was investigated in mixed-breed rabbits. Groups of three male and three female rabbits were given profenofos (purity unknown) as a single dose at 215, 464 and 1000 mg/kg bw. The test substance was applied as a concentrate under occlusive conditions (plaster and aluminium foil) to the shaved back of the rats for 24 h. After 24 h, the plaster and aluminium foil were peeled off and the skin was washed with warm water. Rabbits were observed for 14 days. The study was not conducted in accordance with GLP regulations. Three males and two females at the highest dose (1000 mg/kg bw) and two males and two females at the intermediate dose (464 mg/kg bw) died within 7 days after application of the substance. Twenty-four hours after treatment, rabbits at the intermediate and highest dose showed ataxia, tremor, salivation, loss of postural tone, and sedation. Surviving rabbits recovered

within 12 days. No other details of clinical signs (incidence and observation duration) were provided in the study report. At necropsy, congested organs and bleeding along the gut were seen. There were no adverse effects in rabbits that received 215 mg/kg bw. The dermal LD_{50} was calculated to be 472 (range, 304–733) mg/kg bw (Sachsse, 1974b).

In another study, dermal toxicity was determined in New Zealand White rabbits with intact and with abraded skin. Doses ranged between 72.6 and 2000 mg/kg bw. Profenofos (unknown purity) was applied once as a concentrate and gently massaged into the shaved back of the trunk of test animals and then kept under occlusive conditions for 24 h. In all, 45 females and 50 males were used for this study (five males and five females per group, or five males or five females per group). Gross necropsy was performed after a 14-day observation period. This study was not conducted in accordance with GLP regulations. In addition to some of the symptoms noted in the previous study, signs of activity decrease, constricted pupils, decreased or no defecation, decreased or no urination, diarrhoea, emaciation, lacrimation, ptosis, and small faeces were noted throughout the observation period beginning with day 2 after treatment. Findings on gross necropsy were discoloured organs and again haemorrhagic areas along the gastrointestinal tract. There was no difference between groups with abraded vs intact skin. The acute dermal LD50 for male and female rabbits were 111 (95% CI, 84.8–145) mg/kg bw for rabbits with intact skin and 155 (95% CI, 124–193) mg/kg bw for rabbits with abraded skin. The overall dermal LD50 was 131 (95% CI, 110–156) mg/kg bw (Cannelongo, 1982a).

In a further study, groups of five male and five female albino rabbits were given a single application of profenofos (purity unknown) at a dose of 250, 2010, 2300 and 2600 mg/kg bw applied as a concentrate under a semi-occlusive dressing to the dorsal surface of the trunk for 24 h. Rabbits were observed for 14 days. This study was conducted in accordance with GLP regulations. Beginning on day 1 through 12 days after treatment, one out of five, two out of five, four out of five, five out of five rabbits died at doses of 250, 2010, 2300 and 2600 mg/kg bw, respectively. Clinical signs found were similar to those observed previously and gross examination at necropsy revealed discoloured and gas-distended gastrointestinal tract as well as empty intestinal tract. The dermal LD50 was calculated to be 2560 (range, 2190–2990) mg/kg bw (Kuhn, 1989).

These apparently divergent results in studies of acute dermal toxicity in rabbits were likely to be due to the differences in dressings (occlusive vs semi-occlusive) and application methods used (abraded skin vs massaging the substance into the skin). In rats, there was no indication of increased toxicity via dermal administration.

(c) Exposure by inhalation

Four groups of five male and five female Sprague-Dawley (Crl:CD(SD)BR) rats were exposed (whole body) to aerosol atmospheres of profenofos (purity, 90.5%) at a nominal concentration of 2.23, 2.77, 4.57 or 6.30 mg/l (analytical concentrations of 2.31, 3.10, 4.51, and 6.30 mg/l, respectively) for 4 h. The particle size of the test aerosol was 2.04 μm (mass median aerodynamic diameter, MMAD) with geometric standard deviation of 1.84. Rats in the control group were exposed to air only. At the end of the 14-day observation period, rats were examined by gross necropsy. The study was conducted in accordance with GLP regulations. The mortalities observed at 2.31, 3.10, 4.51, and 6.30 mg/l were three out of ten, four out of ten, five out of ten and ten out of ten, respectively. During exposure, shortly after exposure and after exposure, damp fur, irregular breathing, unkempt fur, yellow/brown stained fur, alopecia, crusty muzzle, salivation, crusty nose, lacrimation, prostration, ataxia, exophthalmos, gasping and tremors were observed among the rats. At necropsy, abnormalities of the lung, eyes, stomach, skin, kidneys, spleen, intestine and external surface were seen. The median lethal concentration (LC50) (4-h) for males and females combined was calculated to be 3.36 mg/l air (Horath, 1982).

Groups of five male and five female Wistar-derived Alpk:AP_fSD rats were exposed to profenofos (purity, 92.1%) at a nominal concentration of 2 mg/l (analytical concentration, 2.2 mg/) by nose-only inhalation for 4 h. The particle size of the test aerosol ranged from 3.01 to 3.51 μm MMAD, with geometric standard deviation in the range of 1.59–1.65. This study was conducted in accordance with GLP regulations. There were no deaths and only transient signs of respiratory irritation (wet fur, salivation, piloerection, haunched posture) were observed in all rats during and immediately after exposure. All males and all except three females had gained weight by the end of the 14-day experimental period and no treatment-related effects were found at necropsy. The LC_{50} (4-h) of profenofos for males and females combined was > 2.2 mg/l air (Rattray, 2004).

In a similar design of study to the above, groups of five male and five female Sprague-Dawley rats were exposed (nose only) to profenofos (purity, 89.0%) at an analytical concentration of 2.03 mg/l for 4 h. The particle size of the test material was 2.5 μm (MMAD). This study was conducted in accordance with GLP regulations. During the 14-day observation period, there were no signs of gross toxicity, abnormal behaviour, mortality and no treatment-related effects on body weight or on gross findings at necropsy. The LC_{50} (4-h) for male and female rats was > 2.03 mg/l air (Durando, 2005c).

(d) Dermal irritation

In a study of primary skin irritation, 0.5 ml of undiluted technical profenofos (purity, 90.4%) was applied under semi-occlusive dressing to the intact skin of New Zealand White rabbits for 4 h. This study was conducted in accordance with GLP regulations. Erythema was present at each observation time until day 21; and oedema was present until day 17 after application. The mean score for erythema formation at 24, 48 and 72 h was 1.94; that for oedema was 1.61. The primary irritation index was 3.3 (Kuhn, 1990b).

Three New Zealand Albino rabbits received 0.5 ml of undiluted profenofos (purity, 92.1%) applied to the skin for 4 h. This study was conducted in accordance with GLP regulations. Very slight to well-defined erythema and very slight oedema were found at the application site in all rabbits 1 h after the end of treatment, but the effects had disappeared within 3 days. The primary dermal irritation index was 1.2 (Merkel, 2004c). Profenofos was not irritating to the rabbit skin.

In a third study, a single application of 0.5 ml of profenofos (purity, 89.0%) was made to the skin of three New Zealand Albino rabbits for 4 h. This study was conducted in accordance with GLP regulations. Well-defined erythema and slight oedema were again noted 1 h after the treatment period. There were no signs of irritation on day 7, but desquamation was observed at all application sites. The primary dermal irritation index was 3.1 and profenofos was classified as moderately irritating to the skin (Durando, 2005d).

(e) Ocular irritation

In a study of primary eye irritation, 0.1 ml of undiluted profenofos (purity, 90.5%) was instilled into the conjunctival sac of the eyes of New Zealand White rabbits. A statement of quality assurance was provided in the study report. However, no statements were made in the report regarding GLP compliance. At 1 h after instillation, the maximum average irritation score was 4.3 and 4.0 for the unwashed and washed eyes. The reactions were limited to a slight transient conjunctivitis (Cannelongo, 1982b).

Similar results were obtained in two more recent studies.

Conjunctivitis was observed in all three New Zealand Albino rabbits 1 h after instillation of 0.1 ml of profenofos (purity, 92.1%). In this study, the conjunctivitis had disappeared within 24 h of

treatment. Corneal opacity was noted in one rabbit, but only at 24 h after treatment and there was no evidence of iritis. Profenofos was classified as non-irritating (Merkel, 2004d).

In the other study, neither corneal opacity nor iritis was noted at any time after treatment with profenofos (purity, 89.0%) Profenofos was classified as mildly irritating to the eye (Durando, 2005e). Both studies were conducted in accordance with GLP regulations.

(f) Dermal sensitization

Guinea-pigs

A study of dermal sensitization was performed on Pirbright White guinea-pigs (Tif:DHP) according to the maximization test protocol of Magnusson & Kligman, which uses addition of Freund adjuvant. This study was conducted in accordance with GLP regulations. The induction phase was performed with an intradermal injection of a 3% solution of profenofos (purity, 91.2%) and an epidermal application of a 30% solution of profenofos, both in peanut oil. The guinea-pigs were challenged with a 5% solution of profenofos in Vaseline. Under these conditions, profenofos showed a sensitization rate of 45% (Winkler, 1996). The Meeting therefore concluded that profenofos may cause sensitization by skin contact.

Mice

The skin sensitization potential of profenofos has also been investigated in the local lymph-node assay. This study was conducted in accordance with GLP regulations. Profenofos (purity, 92.1%) was applied as a 1%, 2.5% or 5% preparation in acetone : olive oil (4 : 1) to the ears of groups of four female CBA/Ca mice. The application was repeated on three consecutive days. Application of the 1% preparation in acetone caused an increase of greater than threefold in the incorporation of [3H-methyl]thymidine into lymph-node cells and profenofos was therefore a sensitizer under the conditions of the study. The groups of mice given the 2.5% and 5% preparations were killed early due to toxicity (Betts, 2005).

In a similar study, the ears of three groups of five female CBA/J mice were treated with 0.5%, 1% or 2% profenofos (purity, 97.3%) in acetone : olive oil (4 : 1) for 3 days. This study was conducted in accordance with GLP regulations. A stimulation index of > 3 was found in mice treated with 2% profenofos, and profenofos was therefore considered to be a sensitizer under the conditions of the study (Kuhn, 2005).

2.2 Short-term studies of toxicity

Erythrocyte acetylcholinesterase activity was found to be significantly more sensitive to profenofos than was brain acetylcholinesterase activity in rats, mice, rabbits, and dogs. However, in no species were any signs of toxicity seen at doses that did not also produce significant inhibition of brain acetylcholinesterase activity. Hence it was concluded that brain acetylcholinesterase activity was the more appropriate indicator of the toxic potential of profenofos.

Rats

Thirteen groups of 25 male and 25 female COBS®CD®F/Cr1BR F344 rats were fed diets containing profenofos (purity, 90.6%) at a concentration of 0 (two groups), 0.01, 0.03, 0.1, 0.3, 1.0, 3.0, 10, 30, 100, 300 or 1000 ppm, equal to intakes of 0, 0.001, 0.0025, 0.009, 0.025, 0.090, 0.24, 0.87, 2.4, 8.4, 22 and 85 mg/kg bw per day, respectively. Five males and five females in each test group and in both control groups were killed after 2 and 4 weeks. The remaining rats in one control group and in the groups at 0.01, 0.03, 0.1, 1, 10, 100 and 1000 ppm were killed after 8 weeks. Rats in the other

control group and in the test groups at 0.3, 3.0, 30 and 300 ppm were killed after 13 weeks. Stability and dietary concentrations were confirmed analytically. Rats were observed daily for mortality and signs of moribundity. Clinical signs, body weights and food consumption were monitored weekly. Blood and urine was collected from rats in all groups at weeks 2 and 4 for haematology and clinical chemistry investigation. Cholinesterase activity was determined in plasma, erythrocytes and brain (2, 4 and 13 weeks), and differences were analysed statistically only for those rats treated for 13 weeks. Histopathological examination was not conducted in this study.

The test compound was stable in the diet over the duration of the feeding period and the concentrations were within 20% of the target. However, at < 1 ppm the concentrations generally exceeded the target.

There were no treatment-related mortalities or clinical signs in any of the test groups. Reduced food consumption and a dose-related growth depression occurred at doses of 100–1000 ppm.. At week 4, the treatment-related depression on growth was slight at 100 ppm (males, 10%; and females, 14%), but marked (males, 45%; and females, 38%) at 1000 ppm. At week 13, reduced body-weight gains were noted at 300 ppm (males, 15%; and females, 17%). Results of haematology, clinical chemistry (without cholinesterase measurements) and urine analysis were unremarkable and did not indicate any relationship to treatment. The activities of plasma and erythrocyte acetylcholinesterase showed a dose-related inhibition in males and females at 10, 30, 300 and 1000 ppm, being more pronounced in females, and generally reached a plateau after 4 weeks. Plasma cholinesterase activity was inhibited by 6–70% in males and 26–90% in females at 10–1000 ppm. The corresponding figures for inhibition of erythrocyte acetylcholinesterase activity were 33–84% in males and 30–84% in males. Slightly lower brain acetylcholinesterase activity was present in the males at 300 ppm (14%) and in the females (11%) at week 13, but this was not statistically significant. At 1000 ppm, brain acetylcholinesterase activity was inhibited by 30% in males and 29% in females. All inhibition data were been calculated based on values for concurrent controls. Necropsies performed during or at the end of the study did not reveal any treatment-related changes that were observable on gross examination.

The NOAEL for inhibition of brain acetylcholinesterase activity was 300 ppm (equal to 22 mg/kg bw per day) on the basis of inhibition of brain acetylcholinesterase activity (males, 30%; and females, 29%) at 1000 ppm (equal to 85 mg/kg bw per day). The NOAEL for other signs of toxicity (excluding cholinesterase activity) was 100 ppm (equal to 8.4 mg/kg bw per day) on the basis of reduced food intake and reduced growth rate at 300 ppm (equal to 22.0 mg/kg bw per day). The study author established a no-observed-effect level (NOEL) of 3 ppm (equal to 0.24 mg/kg bw per day) on the basis of depression of plasma and erythrocyte acetylcholinesterase activity (Piccirillo, 1978). The study was not conducted in accordance with GLP regulations and did not conform to any particular regulatory guideline.

In a 21-day study of toxicity after exposure by inhalation, groups of nine male and nine female RAI albino rats were exposed by nose-only inhalation to profenofos (purity, 89.9%) for 6 h per day, 5 days a week for 3 weeks. The nominal exposure concentrations were 0, 68, 219 and 449 mg/m3. Four males and four females in the control group and the group at 219 mg/m^3 were kept for a 21-day recovery period without further treatment. The particle size of the test aerosol (45–70%) ranged from < 1 to 3 μm (MMAD). Mortalities, appearance, behaviour, signs of toxicity and body weight were monitored daily. Food consumption was recorded weekly. Haematology and blood chemistry measurements were carried out at the end of the treatment and recovery periods. The blood chemistry determinations included cholinesterase activity in plasma and erythrocytes, which was calculated relative to the values for the control group. Rats were killed at the end of the treatment or recovery periods and subjected to a macroscopic examination that included weighing selected organs; tissues were taken for microscopic examination and acetylcholinesterase activity was determined in brain.

All rats of the group at the highest dose and one female of the group at the intermediate dose died during the first week. Clinical signs such as exophthalmos, dyspnoea, tremor, ruffled fur, lateral position and irritation/secretion of the mucous membranes of the eyes and nose were seen after 2 days in the rats at the highest dose (449 mg/m^3). In the group at the intermediate dose and, to a lesser extent also, in the group at the lowest dose, food intake and body-weight gain was impaired, but returned to normal during the recovery period. The results of the haematological and blood chemistry analysis were generally unremarkable for treated and control rats. In rats exposed to 68 and 219 mg/m^3 plasma cholinesterase activity was inhibited by > 35% and > 47% in males and females, respectively. At these same doses, erythrocyte acetylcholinesterase activity was inhibited by > 64% and > 53% in males and females, respectively, and brain acetylcholinesterase activity was similarly inhibited by > 61% and > 50% in males and females respectively. A minor but statistically significant depression in plasma protein concentration was also observed. The effect on erythrocyte acetylcholinesterase activity was reversible, although plasma and brain acetylcholinesterase activity in rats exposed at 219 mg/m3 remained at about 25% below control levels 21 days after the end of treatment. Compound-related pathological findings in the group at the highest dose included acute conjunctivitis, congestion of the nasal mucous membrane, and, in the majority of rats, severe interstitial or purulent keratitis. All surviving rats of the group at 219 mg/m^3 were slightly emaciated. Rats in the group at the lowest dose showed only incidental macroscopic and microscopic findings not related to the inhalation of profenofos (Sachsse, 1977).

On the basis of a significant reduction of brain acetylcholinesterase activity in all treated groups a no-observable-adverse concentration (NOAEC) could not be identified for this study. This study was not conducted in accordance with GLP standards, but the study design was broadly similar to the OECD guideline 412 for a "repeated dose inhalation toxicity: 21 or 14-day study".

Rabbits

Three short-term studies of dermal toxicity were available for review.

In the first study, groups of three male and three female Himalayan rabbits were given a mixture containing profenofos (purity, 89.8%) diluted with a polyethylene glycol and saline (70 : 30, w/w) applied daily to the shaved skin. Doses of 0, 5, 20 and 100 mg/kg bw per day were applied under occlusive dressing during 24 h on 5 days per week for 3 weeks. One male and one female per dose was reserved for a 3-week recovery period. Rabbits were observed daily for clinical signs of toxicity and skin irritation; food consumption and body weight were determined weekly. Acetylcholinesterase activity was measured in plasma and erythrocytes at 4, 10 and 21 days after the start of treatment and, in the rabbit in the recovery group at 5, 11 and 21 days after cessation of treatment. Haematology and other blood chemistry parameters were determined before testing and at the end of the treatment and recovery periods. The rabbits were subjected to a gross necropsy, and selected organs were weighed and processed for microscopic examination. Brain acetylcholinesterase activity was also determined. Cholinesterase activity was calculated as percent of values for the concurrent control group and no statistical analysis was conducted.

All of the rabbits treated with profenofos at 100 mg/kg bw per day died within 6 days after the start of dosing. These rabbits displayed moderate erythema and oedema of the skin, reduced food intake and body-weight gain and various clinical signs of toxicity (dyspnoea, salivation, tremors, ataxia, sedation, and curved position) within 3 days after the start of treatment. Inhibition of plasma cholinesterase activity was increased by 58% and 91% at 5 and 20 mg/kg bw per day, respectively. Inhibition of erythrocyte acetylcholinesterase activity was 20–36% in males and 24–28% in females at 5 mg/kg bw per day and 38–61% in males and 20–49% in females at 20 mg/kg bw per day. At the end of the treatment period, inhibition of brain acetylcholinesterase activity in the rabbits at 5 mg/kg bw per day was 20% and 21% in males and females, respectively. Inhibition was 42% and 30% in males and females, respectively, in rabbits at 20 mg/kg bw per day. Cholinesterase activity recovered rapidly and was close to control levels 21 days after the end of treatment. At necropsy, congestion of the internal organs, particularly liver, was noted in the rabbits at the highest dose. Histopathological examination

of the rabbits at the highest dose showed focal hypertrophy, haemorrhages and fatty changes in the hepatocytes accompanied by necrosis of the liver parenchyma, and slight to moderate atrophy of lymphoid and thymic tissue. Microscopic examination of the skin at the site of dermal application showed oedema, minute haemorrhages, small intradermal pustules, focal acanthosis and parakeratosis. In the rabbits at the lowest and intermediate doses (5 and 20 mg/kg bw per day) the observed changes consisted of slight erythema and oedema at the application site. These effects disappeared during the recovery period. No other unusual findings were observed in these two groups.

Although the number of rabbits used was small, the lowest-observed-adverse-effect level (LOAEL) for inhibition of brain acetylcholinesterase activity was 5 mg/kg bw per day. A NOAEL was not identified in this study (Sachsse, 1976). This study was not conducted in accordance with GLP regulations or any regulatory guideline.

In the second study, profenofos (purity, 92%) suspended in purified water containing 0.5% Tween 80, was applied to the shaved intact skin of groups of five male and five female HAR:PF/CF New Zealand White rabbits. Doses of 0, 0.05, 1 or 10 mg/kg bw per day were applied once under semi-occlusive dressing made of a porous gauze, during 6 h for 5 days per week during a 3-week period. The rabbits were examined daily for clinical signs of toxicity and skin reactions. Body weights and food consumption were recorded weekly. Haematology and clinical chemistry parameters were measured before dosing and at study termination. Inhibition of cholinesterase activity was calculated as percentage of values for the concurrent control group. Gross postmortem examination was conducted at the end of the study; selected tissues were weighed and/or taken for histopathology.

Treatment-related observations among the rabbits at the highest dose included hyperactivity, diarrhoea and soft faeces. Slight local reactions (erythema) were noted in all groups including controls and a well-defined erythema was found in the groups at the intermediate and highest dose during the third week of treatment. Treatment with profenofos at 10 mg/kg bw per day resulted in a 27% and 41% inhibition of plasma cholinesterase activity in males and females, respectively. Erythrocyte acetylcholinesterase activity was statistically significantly inhibited in both sexes, although the inhibition in males (12%) was much less than that in females (28%). Inhibition of brain acetylcholinesterase activity was statistically significant in males of the group at the highest dose (30%) but not in females (15%). Brain acetylcholinesterase activity was also inhibited in rabbits at 1 mg/kg bw per day (15%; not statistically significant). Minor alterations in serum total bilirubin and sodium concentrations as well as gamma-glutamyl transferase activities were also noted in this group.

There were no toxicologically significant effects in the rabbits at the intermediate and lowest dose. The NOAEL was 1 mg/kg bw per day on the basis of inhibition of brain acetylcholinesterase activity in males (30%) and hyperactivity at 10 mg/kg bw per day (Johnson et al., 1984). This study was conducted in accordance with GLP regulations and was also in compliance with the then current version of the OECD guidelines for testing of chemicals.

In a third study of dermal toxicity, groups of 10 male and 10 female New Zealand White rabbits were given repeated dermal applications of profenofos (purity, 91.2%) at a dose of 0, 2.5, 5 or 10 mg/kg bw per day diluted with distilled water containing 0.5% Tween 80, applied under semi-occlusive conditions for 22 days for 5 days per week during weeks 1 and 2 and daily on days 15–22. The exposure period was 6 h per day. Clinical signs, body weight, food consumption and mortality were monitored throughout the study. Ophthalmological examinations were performed before the start and on the last day of treatment. Haematology and clinical chemistry analyses were also carried out on the last day of treatment. These included determination of acetylcholinesterase activity in erythrocytes and brain, and butyrylcholinesterase activity in plasma; inhibition was calculated as percentge of values for the concurrent control group. At termination, rabbits were killed and examined macroscopically; selected organs were weighed and/or examined microscopically.

The treatment produced no significant clinical signs, no effects on mean body weights or on food consumption. No signs of irritation occurred at the application site. No ocular changes were seen and no effects were recorded on haematological parameters. The evaluation of organ weights did not reveal any treatment-related effects. Macroscopic postmortem examination did not indicate any treatment-related changes. Upon microscopic examination, all treated groups were observed to have an increased incidence of acanthosis at the skin application site.

A treatment-related inhibition of cholinesterase activities in plasma, erythrocytes and brain was seen at all doses. At the end of the study there was a statistically significant decrease in plasma cholinesterase activity of 81–86% and in erythrocyte acetylcholinesterase activity of 47–58% at the lowest dose. There was a 33% inhibition of brain acetylcholinesterase in the group at 5 mg/kg bw per day and a 47% inhibition in the group at 10 mg/kg bw per day. The effect was statistically significant at both doses. Brain acetylcholinesterase activity was inhibited by only 14–15% at 2.5 mg/kg bw per day. All other blood chemistry parameters were unaffected by treatment.

The NOAEL for brain acetylcholinesterase activity was 2.5 mg/kg bw per day on the basis of statistically significant inhibition of brain acetylcholinesterase activity in males and females at 10 mg/kg bw per day. The study author concluded that NOAEL for erythrocyte acetylcholinesterase activity could not be determined in this study owing to biologically significant inhibition of erythrocyte acetylcholinesterase activity at the lowest dose (Cantoreggi, 1998). The study was conducted in accordance with GLP regulations and US EPA Health Effects Test Guidelines OPPTS 870.3200 (1996).

Dogs

In a 90-day feeding study, groups of four male and four female beagle dogs were given diets containing profenofos (purity, 94.8%) at a concentration of 0, 2, 20 or 200 ppm (equivalent to 0, 0.05, 0.5 and 5.0 mg/kg bw per day). In addition, one male and one female were added to the untreated control group and to the test group at the highest dose to study recovery during a 28-day period. Stability and test concentrations were confirmed analytically. Body weight and food consumption were recorded weekly, haematology, clinical chemistry and urine analysis was conducted before testing and after 1, 2 and 3 months treatment. At the conclusion of treatment, the dogs were given a complete necropsy; selected organs were weighed and taken for microscopic pathology. Owing to the small sample size, no statistical analysis was conducted. Percentage inhibition of cholinesterase activity was not included in the report, but was been calculated by the Meeting from the tabulated data.

The concentration analyses of the test diets indicated mean concentrations of 96%, 94% and 84% at 2, 20 and 200 ppm, respectively. The stability of the test compound in the diet was demonstrated in a 6-month study in dogs.

There were no treatment-related significant changes observed in food consumption, body weight, haematology, clinical chemistry, urine analysis, ophthalmology, organ weights, organ ratios or macro- and micro-pathology. Plasma cholinesterase activity was inhibited by 32–76% at all dietary concentrations, with no evidence of a dose–response relationship, but returned to normal values during the 28-day recovery period. Inhibition of erythrocyte acetylcholinesterase activity was < 22% in the dogs at 2 ppm. In the group at 20 ppm, inhibition of erythrocyte acetylcholinesterase activity was 13–20% and 30–50% in males and females, respectively. At 200 ppm, there was a 68–80% inhibition of erythrocyte acetylcholinesterase activity in males and females. In dogs in the recovery group, erythrocyte cholinesterase activity remained depressed at about 50% of values before testing (although some recovery was seen with respect to values at 90 days on test). Brain acetylcholinesterase activity in males at 200 ppm was depressed by 21%, but returned to normal at the end of the 28-day recovery period; inhibition of brain acetylcholinesterase activity was < 20% in females at 200 ppm, as well as in males and females at lower dietary concentrations of profenofos. No other treatment-related effects were observed at any dose.

The NOAEL for brain acetylcholinesterase was 20 ppm, equivalent to 0.5 mg/kg bw per day, on the basis of reduced brain acetylcholinesterase activity at 200 ppm, equivalent to 5.0 mg/kg bw per day; the highest dose tested. The NOAEL for other systemic toxicity was 200 ppm, equivalent to 5.0 mg/kg bw per day; the highest dose tested. The study was conducted before development of Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) test guidelines and GLP regulations. The data had not been independently validated (Burtner et al., 1975).

Since a clear NOAEL was not established in the 90-day study, a follow-on 6-month study was conducted. Groups of seven male and seven female beagle dogs were fed diets containing profenofos (purity, 89.3%) at a concentration of 0, 0.2, 2, 100 or 500 ppm, equivalent to 0, 0.0072, 0.05, 2.9 and 14.4 mg/kg bw per day, for 6 months. One male and one female in each dose group was kept for a 4-week recovery period on control diet. The diet analyses were conducted before initiation and throughout the duration of the study. Dogs were observed daily for clinical signs of toxicity. Food consumption was measured daily, while body weight was monitored weekly. Haematology, clinical chemistry (including plasma and erythrocyte cholinesterase activity and plasma and liver carboxylesterase activities) and urine analysis parameters were determined at 4, 9, 13, 18, 22 and 26 weeks, and also in dogs in the recovery group at 31 weeks. Ophthalmoscopy was performed after 26 and 31 weeks. The dogs in the group at 500 ppm were additionally given a battery of tests to examine central and peripheral neural responses (e.g. muscle strength and tone, reflexes). At necropsy, organs and tissues were examined for gross changes, and subsequently by histopathology. Acetylcholinesterase activity was determined in the brain. Data on inhibition of cholinesterase activity were calculated relative to values for dogs in the concurrent control group.

At study initiation, the concentrations of the test material in the diet at 0.02, 2. 100 and 500 ppm were 115%, 74%, 98% and 98%, respectively. The test compound was stable in the diet at –20°C for > 4 weeks.

Besides a transiently-decreased food intake in the males of the group at 500 ppm, no effects were observed with respect to mortality, clinical signs, including special neurological examination, body-weight development, ophthalmoscopy, hearing tests, urine analysis, organ weights, gross examination and histopathology. Slightly decreased erythrocyte parameters (erythrocyte count, haemoglobin concentration and erythrocyte volume fraction) in the group at 500 ppm were the only haematological findings. These haematological findings were not considered to be toxicologically significant since the magnitude of changes were minor, and were within the range for historical controls. Clinical chemistry results indicated that plasma cholinesterase activity was inhibited by 45–64% in both sexes at 2 ppm, 62–79% at 100 ppm and 80–90% at 500 ppm. Erythrocyte acetylcholinesterase activity was inhibited at 100 and 500 ppm by 52–80% and 68–95%, respectively. The inhibition of plasma or erythrocyte acetylcholinesterase activity was essentially unchanged from week 4 until the end of the treatment period. Plasma and liver carboxylesterase activities were decreased in males of the groups at 100 and 500 ppm. The effects on plasma and erythrocyte acetylcholinesterase activity showed a tendency to reverse during the 4-week recovery period. Brain acetylcholinesterase activity was inhibited by only 5% at 2.0 ppm in males; in females, inhibition was 8%, 10%, 11%, and 5% at dietary concentrations of 0.2, 2.0, 100 or 500 ppm, respectively. No data for the recovery group were available for inhibition of brain acetylcholinesterase activity.

The potential oculotoxic hazard of profenofos was assessed further through re-evaluation of relevant data from this 6-month study in dogs. No ophthalmological findings related to treatment were observed. Histological examination of the eyes was unremarkable and some of the findings were confined to dogs in the control group. Re-evaluation of haematology and blood chemistry data confirmed the results outlined above, that profenofos is not toxic to the ocular or haematological system in dogs.

The NOAEL for inhibition of brain acetylcholinesterase activity was 500 ppm, equivalent to 14.4 mg/kg bw per day; the highest dose tested. The study was not conducted in accordance with GLP regulations and there was no claim of compliance with a regulatory guideline. However, a protocol was available and the study design was consistent with OECD guideline 409 (Gfeller, 1981).

In another study, groups of four male and four female beagle dogs were given gelatin capsules containing profenofos (purity, 91.2%) at a dose of 0, 0.015, 0.05, 1 or 12.5 mg/kg bw per day mixed with lactose for 1 year. Six mixtures of each concentration were prepared for the 1-year period and were stored at 0–5°C. Clinical signs and food consumption was monitored daily and the dogs were weighed weekly. Eye examinations were conducted and tonometry measurements made before testing, and at weeks 7 (tonometry only), 13, and 26, and towards the end of the treatment period. Neurological, clinical and haematological investigations were made at weeks 13, 26 and 52. This included determination of acetylcholinesterase activity in erythrocytes and butyrylcholinesterase activity in plasma. The dogs were given a gross examination at necropsy and selected organs were weighed. A microscopic examination was also conducted and acetylcholinesterase activity was determined in brain. Cholinesterase activity was analysed statistically and inhibition calculated relative to the values measured before testing.

The analyses of dietary concentration were performed at higher concentrations. The results showed that the mean concentrations at 1000 and 50 000 ppm (29 and 1450 mg/kg bw per day, respectively) were 90% and 97.6% of the nominal concentrations, respectively. The test compound was homogeneously distributed and was stable in lactose powder (bulk or in capsules) for 10 weeks under the conditions of the study.

There were no treatment-related deaths in this study. No treatment-related changes were observed in behaviour, food consumption, eye examination, intraocular pressure, neurological examination, urine analysis, organ weights, and macroscopic examinations at necropsy. Mean body-weight gain was slightly depressed at 12.5 mg/kg bw per day, mainly due to results for one female.

Haematology parameters, namely erythrocyte count, haemoglobin concentration and erythrocyte volume fraction, were reduced in males and females at 12.5 mg/kg bw per day and in males at 1 mg/kg bw per day. Mean erythrocyte volume was elevated in males at 12.5 mg/kg bw per day. At weeks 26 and 52, a slightly higher platelet count was recorded in males at 1 and 12.5 mg/kg bw per day. The changes in the haematological parameters were not considered to be toxicologically significant since there was no clear dose–response relationship and the magnitude of changes observed were within the range for historical controls. Small changes in blood chemistry were noted in the groups at 1 or 12.5 mg/kg bw per day. They included reduced concentrations of plasma protein and albumin and, as a result, a reduced albumin : globulin ratio. Reductions in calcium concentration paralleled those of plasma albumin owing to its calcium-binding properties. Plasma glucose concentrations were decreased, and alkaline phosphatase activities increased. Changes in the clinical chemistry parameters were not considered to be toxicologically relevant.

There was a 54–68% inhibition of erythrocyte acetylcholinesterase activity in dogs at 1 mg/kg bw per day and an 82–86% inhibition at 12.5 mg/kg bw per day. At both doses the inhibition was statistically significant. There was no sex difference in cholinesterase inhibition and no significant change in inhibition after the first determination in week 13. There was also a dose-dependent inhibition of plasma cholinesterase activity in all groups; at the lowest dose; the inhibition was 26–55% in males and 13–42% in females, although the group mean differences did not achieve statistical significance. Brain acetylcholinesterase activity in the group at the highest dose was slightly (not statistically significantly) lower than that of dogs in the control group (males, 11%; and females, 6%). Neurotoxic esterase activity was not affected by the treatment.

Microscopic examination revealed an increased number of binucleated perilobular hepatocytes in males and females at 1 and 12.5 mg/kg bw per day, accompanied by hyperplasia of bile-duct epithelium in the males. Concentrations of bile pigments were increased in the convoluted renal tubules in dogs at 12.5 mg/kg bw per day. These pathological findings (Table 2) were minimal in severity and were not observed in the 90-day or 6-month studies of toxicity in dogs given similar doses. In addition, they were not associated with any biological correlates. Therefore, a NOAEL was not identified on the basis of these findings.

Table 2. Histopathological findings in dogs fed capsules containing profenofos for 1 year

Finding	Severity	No. of dogs affected (n = 4)[a]									
		Dose (mg/kg bw per day)									
		Males					Females				
		0	0.015	0.05	1.0	12.5	0	0.015	0.05	1.0	12.5
Kidney											
Deposition of bile pigment	Grade 1	1	3	1	1	1	2	0	2	2	2
	Grade 2	1	0	1	1	1	0	2	0	0	1
	Grade 3	0	0	0	0	3	0	0	0	0	0
	Total[b]	2	3	2	2	4	2	2	2	2	3
	Average[c]	—	1.5	1.0	1.5	2.0	1.0	2.0	1.0	1.0	1.3
Liver											
Increase in binucleated hepatocytes	Grade 1	0	0	0	3	0	0	0	0	4	0
	Grade 2	0	0	0	0	1	0	0	0	0	2
	Grade 3	0	0	0	0	3	0	0	0	0	2
	Total[b]	0	0	0	3	4	0	0	0	4	4
	Average[c]	—	—	—	1.0	2.8	—	—	—	1.0	2.5
Hyperplasia of bile duct	Grade 1	0	0	0	1	1	0	0	0	0	0
	Grade2	0	0	0	0	1	0	0	0	0	0
	Total[b]	0	0	0	1	2	0	0	0	0	0
	Average[c]	—	—	—	1.0	1.5	—	—	—	—	—

From Altmann (1999)
[a] Average values for four dogs.
[b]Total number of dogs with tissues affected
[c]Average grade.

The NOAEL was 12.5 mg/kg bw per day (Altmann, 1999). The study was conducted in accordance with GLP regulations and followed the US EPA FIFRA pesticide assessment guidelines, subdivision F (1982), section 83-1 and OECD guideline No. 452 (1981).

The results of three studies of toxicity in dogs indicated that brain acetylcholinesterase activity was significantly inhibited in males at 5 mg/kg bw per day in the 90-day study, but not in males or females at 2.9 or 14.4 mg/kg bw per day in the 6-month study, or at 1 or 12.5 mg/kg bw per day (the highest dose tested) in the 1-year study. Hence, the overall NOAEL for inhibition of brain acetylcholinesterase activity, in these three studies in dogs was 2.9 mg/kg bw per day.

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a 2-year study of carcinogenicity, groups of 65 male and 65 female HaM/ICR Swiss, CR CD-1 albino mice were given diets containing profenofos (purity, 90.6%) at a concentration of 0, 1, 30 or 100 ppm (equal to ingested doses of 0, 0.14, 4.5 and 14.2 mg/kg bw per day in males and 0, 0.19, 5.77 and 19.18 mg/kg bw per day in females, respectively) for 85 weeks (males) or 96 weeks (females). The test diets were prepared weekly and shipped to the sponsor for analysis. However, the results of analyses were not provided by the sponsor. Mortality was checked daily, while clinical signs, individual body weights and food consumption were measured once every 4 weeks. The

presence of nodules or masses was checked by palpation monthly during the first 41 weeks of the study and weekly thereafter. Five males and five females per group were killed at week 53 and subjected to gross necropsy. All surviving mice were examined by gross necropsy at the termination of feeding. Selected organs from all necropsied mice were weighed and examined histopathologically. Determinations of plasma, erythrocyte, and brain acetylcholinesterase activity were carried out on mice killed at week 53 and on five or six males and ten females per group at the end of the study. Inhibition of cholinesterase activity was calculated using the values for concurrent controls.

Treatment did not adversely affect survival. Survival of male mice treated with profenofos at 0, 1, 30 and 100 ppm was 63%, 55%, 65%, 63%, respectively. The corresponding figures for female mice were 65%, 70%, 75% and 78%, respectively. There were no indications of a treatment-related effect with respect to clinical signs of toxicity, body weights, food consumption, incidence of tumours, gross pathology, or histopathology. In treated mice, plasma cholinesterase activity was inhibited by 46–76% at 100 ppm and by 38–68% at 30 ppm. Erythrocyte acetylcholinesterase activity was also statistically significantly inhibited by 66–74% and 49–68% in mice at 100 and 30 ppm respectively. There was no sex difference in inhibition of either plasma or erythrocyte acetylcholinesterase activity. In females, there was a statistically significant inhibition of brain acetylcholinesterase activity (25%) at termination of the group at 100 ppm. Brain acetylcholinesterase activity was also decreased in male mice at 100 ppm at week 53 (15% inhibition); but there was an increase in acetylcholinesterase activity in this group at termination. Hence, the effect in males at week 53 was considered not to be biologically relevant.

The results of this study suggested that profenofos is not carcinogenic in mice. The NOAEL was 30 ppm, equal to 4.5 mg/kg bw per day, on the basis of inhibition of brain acetylcholinesterase activity at 100 ppm, equal to 14.2 mg/kg bw per day, in female mice (Burdock, 1981b).

Rats

In a long-term study of toxicity, groups of 60 male and 60 female Fischer 344 albino rats were fed diets containing profenofos technical (purity, 90.6%) at a concentration of 0, 0.3, 10 or 100 ppm (equal to 0, 0.017, 0.56, and 5.7 mg/kg bw per day in males and 0, 0.02, 0.69, and 6.9 mg/kg bw per day in females) for 2 years. An additional 10 males and 10 females were also included in the control group and the group at the highest dose, respectively. Of the latter, five males and five females per group were killed at 52 weeks (interim kill), and five males and five females per group were placed on control feed after 52 weeks so that recovery could be investigated; these rats were then killed during week 63 (recovery group). The study report stated that the test diets were shipped weekly for analyses. However, the results were not available for review. The rats were observed daily for morbidity and moribundity. Body weights, food consumption and clinical signs were recorded weekly for the first 14 weeks and monthly thereafter. The rats were examined and palpated weekly for tissue masses. Blood and urine were collected for clinical chemistry and haematological investigations from 10 males and 10 females at weeks 13, 26, 52, 78 and 105 and from five males and five females in the recovery group after 63 weeks. Plasma and erythrocyte acetylcholinesterase activity was determined at 13, 26, 52, 78, and 105 weeks (main study groups) and in the recovery group at week 57. Brain acetylcholinesterase activity was determined at interim and terminal kill at weeks 53 and 105. Inhibition of cholinesterase activity was calculated in comparison with concurrent control values. Gross necropsy was performed for all rats. Selected organs from all rats necropsied were weighed and examined histopathologically.

There was no treatment-related increase in mortality. Survival in groups of male rats at 0, 0.3, 10 and 100 ppm was 85%, 90%, 80% and 83%, respectively. The corresponding survival in female rats at 0, 0.3, 10 and 100 ppm was 78%, 72%, 77% and 68%, respectively. No clinical signs of toxicity were observed, and body weights were not affected at any dose. However, at the highest dose, there was an increase in food consumption in females. At the highest dose, there was an increase in relative thyroid weight in males (in rats at the interim kill and in the recovery group but not at terminal kill), an increase in thyroid gland perifollicular-cell hyperplasia in males (4 out of 70 in the control group vs 10 out of 70), and an increase in liver neoplastic nodules in females (control group,

1 out of 70; lowest dose, 3 out of 60; intermediate dose, 2 out of 60; and highest dose, 6 out of 70). These histopathological findings were not considered to be treatment-related, or to be suggestive of an oncogenic effect. No increase in the incidence of liver carcinomas occurred.

Inhibition of plasma cholinesterase activity in rats in the group at 100 ppm was statistically significant at all sampling times and ranged from 30% to 62% in males and 50% to 62% in females. There was a statistically significant inhibition of plasma cholinesterase activity at some sampling times in males at 0.3 ppm and in males and females at 10 ppm. However, inhibition never exceeded 20% and 28% in rats at 0.3 ppm and 10 ppm, respectively. There was a statistically significant inhibition of erythrocyte acetylcholinesterase activity that ranged from 58% to 71% in rats at 100 ppm and from 12% to 31% in rats at 10 ppm. Inhibition of erythrocyte acetylcholinesterase activity was noted at some time

Table 3. Results of studies of genotoxicity with profenofos

End-point	Test object	Concentration	Purity (%)	Results	Reference
In vitro					
Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, *Escherichia coli* WP2 *uvr*A	312.5–5000 µg/plate in DMSO	90.7	Negative ± S9	Ogorek (1991)
Gene mutation	Mouse lymphoma L5178Y/ *Tk*+/-cells	0.078–0.625 µg/ml in DMSO	91.8	Negative ± S9	Strasser (1982)
Chromosomal aberration[a]	Chinese hamster ovary cells (CCL 61)	4.69–75 µg/ml in DMSO	90.7	Negative ± S9	Strasser (1990)
Chromosomal aberration[b]	Chinese hamsters	13–52 mg/kg bw in CMC	88.1	Negative	Hool (1981)
Micronucleus formation[c]	Mouse (Tif:MAGf) bone marrow	50–200 mg/kg bw	90.7	Negative	Hertner (1990)
		0–216 mg/kg bw	72	Equivocal	El Nahas et al. (1988)
Dominant lethal mutation	Male mice (NMRI)	35 and 100 mg/kg bw	NR	Negative	Fritz (1974a)
Mitotic gene conversion, mitotic crossing over, reverse mutation	*Saccharomyces cerevisiae* D7	12.5–500 µg/ml without activation, 640–1000 µg/ml with activation	90	Negative	Arni (1982)
Mitotic disjunctione	*Saccharomyces cerevisiae* D61.M	39.06–10000 µg/ml with and without activation	91.8	Negative	Hool (1986)
Unscheduled DNA synthesis	Rat hepatocytes	0.02–2.91 µg/ml in DMSO	91.8	Negative	Puri (1982a)
Unscheduled DNA synthesis	Human fibroblasts	0.46–58.2 µg/ml in DMSO	91.8	Negative	Puri (1982b)

CMC, caboxymethyl cellulose; DMSO, dimethyl sulfoxide, NR, not reported; S9, 9000 × g supernatant prepared from Arochlor-induced rat liver.

[a] Cells were evaluated after: (i) 3-h incubation without metabolic activation at 18.75–75 µg/ml; (ii) 3-h incubation with metabolic activation at 4.69–18.75 µg/ml; and (iii) 24-h incubation without metabolic activation at 9.38–37.5 µg/ml.

[b] Bone-marrow cells were examined for anomalies such as single Jolly bodies, polyploid cells, fragments of nuclei in erythrocytes, micronuclei in erythroblasts and leukopoietic cells.

[c] Micronuclei were counted in bone-marrow erythrocytes.

[d] A formulation was tested that contained unknown formulation components. Under the experimental conditions (small number of animals, only one sex, low reproducibility, unusually high background values, no overall clear dose–response relationship, only one sampling time in some tests), some mutagenic activity of the formulation could not be excluded but the result was not confirmed in guideline-compliant studies.

[e] Monosomic colonies resulting from chromosomal loss were recognized as leucine-requiring white colonies that were resistant to cycloheximide.

intervals at 0.3 ppm, but never exceeded 20%. The effects on plasma and erythrocyte acetylcholinesterase activity were fully reversible after 4 weeks of recovery following a 52-week dosing period. Brain acetylcholinesterase activities were statistically significantly inhibited in females at 100 and 10 ppm at week 105, but the inhibition was only 12 and 9% respectively. Statistical re-evaluation of erythrocyte acetylcholinesterase activities at 0.3 ppm with values for the study controls after combination with values for historical controls revealed that these differences were not statistically significant.

Although the highest dose did not produce overt toxicity, the dosing was considered adequate for testing for carcinogenicity because there was significant inhibition of plasma and erythrocyte cholinesterase activity, minimal toxicity in the thyroid and liver and minimal inhibition of brain acetylcholinesterase activity in females at 10 and 100 ppm at week 105. In addition, the steepness of the dose–response relationship characterizing the effects of this compound on cholinesterase inhibition posed a limitation on the ability to test for the effects of profenofos at higher doses.

The results of this study lead to the conclusion that profenofos is not carcinogenic to rats. The NOAEL was 100 ppm, 5.7 mg/kg bw per day, the highest dose tested. The report author concluded that the NOEL was 0.3 ppm, 0.017 mg/kg bw per day, on the basis of inhibition of plasma and erythrocyte acetylcholinesterase activities at 10 ppm, 0.56 mg/kg bw per day (Burdock, 1981a).

2.4 Genotoxicity

In previous evaluations, the Meeting had concluded that after reviewing the results of short-term tests in vitro and in vivo, there was no evidence of genotoxicity. The results of additional new studies, in which the genotoxic potential of profenofos was investigated in eukaryotic and prokaryotic systems in vivo and in vitro, did not alter the previous conclusions of the Meeting. The results are listed in Table 3.

2.5 Reproductive toxicity

(a) Multigeneration studies

In a study of reproductive toxicity, Crl:CD®(SD)BRVAF/Plus™ rats received diets containing profenofos (purity, 92%) at a concentration of 0, 5, 100 or 400 ppm (equivalent to 0, 0.4, 7 and 35 mg/kg bw per day) for two generations. Administration started when rats of the P generation were age 43 days and was continued until termination. Rats were observed daily for clinical signs. Body weights and food consumption were determined weekly. After treatment for 10 weeks, rats were randomly paired within each group for up to 21 days to yield the F_1 generation. Stability, homogeneity and dietary concentrations were confirmed analytically. Body weights and food consumption were determined on days 0, 6, 13 and 20 of gestation and on days 0, 4, 7, 14 and 21 of lactation. Litter size, number of live and dead pups, individual sexes, weights, and external observations were recorded for pups on the same days of lactation. On day 4 of lactation, litters were culled to four males and four females wherever possible. Culled pups underwent a soft tissue examination with the focus on brain and heart. F_1 pups were weaned at age 21 days. Within each group, rats were randomly selected to continue on treatment as parent animals of the F_2 generation. They underwent the same study phases as P animals. All non-parental F_1 weanlings and all F_2 weanlings were examined as indicated for the culled pups. Adult rats were necropsied and reproductive tissues were examined histologically.

The mean concentrations of the test compound in the diet were 5.02, 99.3 and 398 ppm for the diets at 5, 100, and 400 ppm , respectively. Profenofos was homogeneously distributed in the diet. It was stable in the diet (bulk storage) at 4°C or at room temperature for 35 days and in feed jars at room temperature it was stable for 16 days. At 400 ppm, reduced body weights (4–11% decrease), body-weight gains (6–16% decrease) and food consumption (7–15% decrease) were noted in male and female parental animals. At 400 ppm, reduced pup body weights (2–9% decrease) and

body-weight gains (3–10% decrease) on days 14 and 21 of lactation. There were no treatment-related clinical signs, necropsy findings or histopathological observations at any dose. Reproduction was not affected at any dose. There were no effects on mating behaviour, duration of gestation, the number of litters with live-born pups, the total number of pups per litter, pre-weaning losses, survival indices and other reproductive indices. No macroscopic findings were noted in pups.

The NOAEL for reproductive toxicity was 400 ppm, equivalent to 35 mg/kg bw per day. On the basis of reduced body-weight gains and food consumption at 400 ppm, the NOAEL for parental and pup systemic toxicity was 100 ppm (7 mg/kg bw per day) (Minor & Richter, 1994).

In an earlier three-generation study of reproduction in CD strain Charles River albino rats, dietary treatment with profenofos (purity, 95.5%) at 0, 0.2, 1.0 and 20 ppm (equivalent to 0, 0.01, 0.05, and 1.0 mg/kg bw per day) did not affect brain acetylcholinesterase activity, reproductive performance, or the development and survival of the offspring through three generations. The NOAEL for reproductive effects was at least 20 ppm, equal to 1 mg/kg bw per day; the highest dose tested. This study was conducted before development of FIFRA guidelines. The data had not been independently validated (Phillips, 1978).

(b) Developmental toxicity

Rats

In a study of developmental toxicity, groups of 25 pregnant Sim:(SD)BR Sprague-Dawley rats were given profenofos (purity, 88.0%) at a dose of 0, 10, 30, 60, 90, or 120 mg/kg bw per day by oral gavage on days 6–15 of gestation. The study was not conducted in accordance with GLP regulations and there was no claim for compliance with any particular guidelines. However, the study design conforms to the OECD guideline No 414. The rats were examined daily for changes in clinical signs and were weighed on days 0, 6 to 15 and 20 of gestation. Food consumption was measured on days 6, 13 and 20 of gestation. On day 21 of gestation, all dams were killed and fetuses were delivered by caesarean section. Uterine contents were examined and fetuses were weighed, sexed and examined for abnormalities. Intracranial structures were examined in approximately half of the fetuses from each litter.

Maternal toxicity was evident at a dose of 120 mg/kg bw per day. Four rats died or were terminated and food consumption was reduced on days 6–13. Various clinical signs of toxicity (e.g. hypoactivity or tremors, ocular porphyrin discharge, dyspnoea, diuresis, and hypothermia) were noted. Two of the four dams that died displayed these clinical signs, while the other two did not. In addition, two of the dams that died also showed scattered haemorrhages in the stomach upon gross necropsy. One rat in the group at 60 mg/kg bw per day also died, but there were no clinical signs of toxicity at doses of up to 90 mg/kg bw per day. Measures of prenatal toxicity such as litter size, percentage of live fetuses, number of resorbed fetuses, number of dead fetuses and the mean sex ratio were not significantly different between the control and treated groups. Malformations and developmental variants observed in fetuses in the control group and those in groups receiving profenofos were within the normal range.

Profenofos was not embryotoxic or teratogenic in rats given doses of up to 120 mg/kg bw per day. The NOAEL for maternal toxicity was 90 mg/kg bw per day on the basis of mortality and clinical signs of toxicity seen at 120 mg/kg bw per day. The NOAEL for developmental toxicity was 120 mg/kg bw per day, the highest dose tested (Harris, 1982).

Another study of developmental toxicity in rats given profenofos at lower doses arrived at similar conclusions. In this study, 23–25 pregnant JCL-SD rats were given profenofos (purity, 95.8%) at a dose of 0, 18, 35, or 70 mg/kg bw per day via intubation on days 7–17 of gestation. The doses selected for testing were based upon the results of a preliminary range-finding study in which a dose of 140 mg/kg bw per day caused death in five out of six treated rats during days 8–15 of gestation.

The study was not conducted in accordance with GLP regulations and no compliance with guidelines was claimed.

Treatment with profenofos was associated with increases in body weight in the dams at doses of 35 and 70 mg/kg bw per day on days 20 and/or 21, increased water consumption on days 17 and 20–21, respectively, and an increase in food consumption at 70 mg/kg bw per day on days 14–21. Although increases in the weights of several organs (heart, spleen, liver, and right kidney) were seen at 70 mg/kg bw per day, these were small in magnitude. No treatment-related changes in mortality or behaviour were observed, and no abnormal findings were observed at gross necropsy. There were no adverse effects on the offspring with respect to resorptions, sex ratios, placental weights, body weights and lengths, or distribution of fetuses within the uterine horns. External and visceral examinations of fetuses were unremarkable. Skeletal examination of fetuses showed increased incidences of progeny with holes in the xiphoid at the intermediate and highest doses (controls, 0%; lowest dose, 0%; intermediate dose, 18.8%; and highest dose, 15.6%) and delayed ossification of vertebral arches at the highest dose (controls, 8.8%; lowest dose, 6.7%; intermediate dose, 0.5%; and highest dose, 26.7%). No data for historical controls were provided and it could not be determined whether the findings were all from one litter or from multiple litters.

The NOAEL for maternal and developmental toxicity was 70 mg/kg bw per day, the highest dose tested (Sugiya et al., 1982).

In an earlier study conducted before establishment of FIFRA test guidelines, profenofos (technical grade; purity unspecified) was administered to groups of 20–27 pregnant rats (strain unspecified) at a dose of 0, 10, 30, or 60 mg/kg bw per day by oral gavage on days 6–15 of gestation. On day 21 of gestation, all dams were killed and fetuses were delivered by caesarean section. Maternal toxicity was indicated by a slight decrease in food consumption at 30 mg/kg bw per day and a marked decrease in food consumption at 60 mg/kg bw per day during the period of treatment. No other adverse effects occurred in the dams. Similarly, profenofos did not appear to affect embryonic or fetal development and no teratogenic effects were observed at any dose tested.

The NOAEL for maternal toxicity was 30 mg/kg bw per day on the basis of markedly decreased food consumption seen at 60 mg/kg bw per day. The NOAEL for fetotoxicity/teratogenicity was 60 mg/kg bw per day; the highest dose tested (Fritz, 1974b).

Rabbits

In a study of developmental toxicity, groups of 16 pregnant New Zealand White rabbits were given profenofos (technical grade; purity, 90.8%) at a dose of 0, 30, 60, 90, and 175 mg/kg bw per day by gavage on days 6–18 of gestation. Rabbits were observed daily for clinical signs; body weights were measured on days 0, 6, 9, 12, 15, 18, 25 of gestation and before caesarean section. On day 30 of gestation, all does were euthanized and fetuses delivered by caesarean section. Uterine contents were examined and the fetuses were examined for external abnormalities. Visceral dissections and skeletal evaluations were performed on all fetuses. The doses of profenofos selected for testing were based upon the results of a preliminary range-finding study in which doses up to 150 mg/kg bw per day did not produce any signs of toxicity. The study was not conducted in accordance with GLP regulations and there was no claim for compliance with any particular guidelines, although the study design conformed to the OECD guideline No. 414.

Treatment with profenofos was associated with anorexia in all groups, but particularly in the group at 175 mg/kg bw per day. Clinical signs of toxicity (including diarrhoea, soft stools, oral/perianal discharges) were noted at 175 mg/kg bw per day and nine of the does at 175 mg/kg bw per day died. Many of the does that died exhibited the above clinical signs of toxicity as well as signs of pin-point stomach haemorrhages and yellow-discoloured areas in the mesentery in the gastric region upon gross necropsy. No statistically significant differences were observed between the control and

treated groups for body-weight gains during gestation or mean number of corpora lutea. No significant differences were observed for the following measures of prenatal toxicity: mean number of implantations, litter size, fetal body weight or embryolethality. No significant differences were detected between the control group and groups receiving profenofos for malformations or variations.

The NOAEL for maternal toxicity was 30 mg/kg bw per day on the basis of reduced maternal-weight gain and food consumption seen at doses of 60 mg/kg bw per day and greater. The NOAEL for developmental toxicity was 175 mg/kg bw per day; the highest dose tested (Holson, 1983).

Previously, the 1990 JMPR had reviewed a study of developmental toxicity in Chinchilla rabbits given profenofos at a dose of 0, 5, 15 or 30 mg/kg bw per day. In this study, the NOAEL for parental and developmental toxicity was greater than 30 mg/kg bw per day, the highest dose tested (Fritz, et al., 1979).

2.6 Special studies

(a) Acute neurotoxicity

This study was conducted in two phases to determine the NOAEL for clinical signs and inhibition of cholinesterase activity after single doses of profenofos (purity, 92%) by oral gavage. In each phase, test groups consisted of five males and five females per group (Crl:CD®BR/VAF/Plus®). The aim of phase 1 was to identify a NOAEL for clinical signs and body-weight effects, while the aim of phase 2 was to identify a NOEL/NOAEL for effects on plasma, erythrocyte and brain acetylcholinesterase activity.

In phase 1, undiluted profenofos (purity, 92%) was administered at a dose of 100, 200, 300 or 400 mg/kg bw in female rats and 100, 200, 400, 600 or 800 mg/kg bw in male rats. There was no control group. Clinical signs were recorded 1 2, 4 and 8 h after treatment and daily thereafter. Mortalities were checked twice per day, body weights were determined on days 0, 7 and 14 or at death when survival exceeded 1 day. All surviving rats were necropsied on day 14 and tissues with macroscopic lesions were retained.

Three males at 800 mg/kg bw, one male at 600 mg/kg bw and one female at 400 mg/kg bw died within the first 5 days. Body-weight gains appeared to be unaffected by treatment with profenofos. Clinical signs of toxicity such as hypoactivity, red-stained face, dark-stained urogenital area, soft stool or few faeces were seen at doses of 200 mg/kg bw and greater. Rats surviving to study termination exhibited no macroscopic lesions. Test material-related findings observed in the males at 800 mg/kg bw were red ocular discharge, erosion/ulceration of the stomach, nasal discharge and diffuse reddening of the stomach. One male at 600 mg/kg bw showed dark fluid in the intestinal tract and one female at 400 mg/kg bw showed yellow perineal staining and dark-red eroded areas of the stomach and duodenum.

The NOAEL for single oral application of profenofos was 100 mg/kg bw on the basis of clinical signs at 200 mg/kg bw and greater.

In phase 2, groups of five male and five female rats were given a single dose of profenofos (purity, 92%) at 0, 0.1, 0.5, 25, 100, or 400 mg/kg bw in corn oil. Clinical signs were recorded 1, 2 and 4 h after treatment. Rats were weighed approximately 4 h after dosing, blood samples were taken for determination of plasma and erythrocyte cholinesterase activity and all rats were necropsied. Inhibition of cholinesterase activity was calculated relative to values for the respective control group. Tissues with macroscopic lesions were examined histologically. The brain of each rat was flash-frozen for evaluation of brain acetylcholinesterase activity. The study was conducted in accordance with GLP regulations and followed the US EPA FIFRA subdivision F, addendum 10-neurotoxicity series 81-1) guidelines.

The only clinical sign was soft stool, observed in one or a few rats in each group. Findings at necropsy were regarded as incidental. Plasma cholinesterase activity was significantly reduced (71–98%) in males and in females at 25, 100 or 400 mg/kg bw. Erythrocyte acetylcholinesterase

activity was inhibited by 21% and 30% in males at 100 or 400 mg/kg bw, although only at 400 mg/kg bw was the inhibition statistically significant. There was a statistically significant and dose-dependent inhibition of cholinesterase activity in erythrocytes of females at 25, 100 or 400 mg/kg bw, which ranged from 31% to 46%. Brain acetylcholinesterase activity was significantly inhibited by 37% and 43%, respectively, in males and females at 400 mg/kg bw.

The NOAEL was 100 mg/kg bw on the basis of the inhibition of brain acetylcholinesterase activity at 400 mg/kg bw. The report author considered the NOAEL for erythrocyte acetylcholinesterase in males to be 25 mg/kg bw because there was a 21% inhibition in rats at 100 mg/kg bw (Glaza, 1994).

In another study, groups of 15 male and 15 female Hsd:Sprague Dawley SD™ rats were given profenofos (purity, 92%) at a dose of 0, 95, 190 and 380 mg/kg bw once by gavage. Rats int eh positive-control group were given a single dose of either propoxur (6 mg/kg bw) or triadimefon (males, 100 mg/kg bw; and females, 150 mg/kg bw). Rats were observed twice per day for clinical signs and mortality and given a physical examination each week. Body weight and food consumption were also monitored weekly. Ten males and ten females per group were monitored in a functional observation battery (FOB) and observed for motor activity in a figure-of-eight maze. These tests were performed 1 week before exposure to profenofos, approximately 4–6 h after dosing (estimated time of peak effect) and on days 7 and 14 after dosing. Blood samples from the remaining five males and females per group were taken at the time of peak effect (approximately 4 h after dosing) and on day 14 for determination of plasma and erythrocyte acetylcholinesterase activity. In addition, brain acetylcholinesterase activity was determined in these rats 14 days after exposure. At necropsy, 10 males and 10 females per group were examined in a special histopathological investigation; tissues were fixed by whole-body perfusion and the brain, spinal cord, peripheral nerves, skeletal muscles and the eyes with the optic nerve were examined in detail. Further organs were examined if gross lesions occurred. This study was conducted in accordance with GLP regulations and the US EPA FIFRA subdivision F guideline, addendum 10-neurotoxicity series 81, 82 and 83.

One male in the group at 380 mg/kg bw died on study day 2. A transient decrease in body weight, cumulative body-weight gain, food consumption and food conversion efficiency was recorded in the first week after exposure in males in the groups at 190 and 380 mg/kg bw, but was compensated for by higher values in the second week. Food consumption was reduced in females at 380 mg/kg bw in week 1, but increased in week 2. As a result, food conversion efficiency was increased in females in week 2. Effects on the autonomic nervous system (diarrhoea, lacrimation, slight impairment of respiration, and miosis), bizarre behavioural effects, neuromuscular effects (ataxia, abnormal gait, impaired reflexes etc.) and effects on the central nervous sytsem (tremors, altered posture, and ease of handling) were only observed in rats of the group at 380 mg/kg bw at the time of peak effect. Motor activity of these animals was reduced at this time-point, but was not apparent at 7 and 14 days after treatment. At the time of peak effect in the group at the highest dose, no effects on the FOB and motor activity tests were observed in the two groups at the lower doses. There was an 84–97% inhibition of plasma cholinesterase activity and 68–96% inhibition of erythrocyte acetylcholinesterase activity across all three groups. The inhibition of erythrocyte cholinesterase activity was statistically significant at all doses. At the 14-day time-point, erythrocyte acetylcholinesterase activity showed some recovery while plasma cholinesterase activity had returned to control levels. Brain acetylcholinesterase activity was not inhibited at the 14-day time-point. All necropsy observations as well as all microscopic findings were interpreted as incidental. There was no evidence of neuropathological alterations attributable to treatment with profenofos in any of the tissues examined.

The dose of 380 mg/kg bw was toxic with rats showing autonomic and functional changes (on FOB) consistent with inhibition of cholinesterase activity. The dose of 190 mg/kg bw was identified as the NOAEL for central nervous system effects. Pathological examination revealed no evidence of compound-related neurotoxicity at any dose (Pettersen & Morrissey, 1993, 1994a).

(b) Short-term studies of neurotoxicity

In a 90-day study of neurobehavioural toxicity, groups of 15 male and 15 female Hsd:Sprague-Dawley rats were given diets containing profenofos (purity, 88.4%) at a concentration of 0, 30, 135 or 600 ppm (equal to 0, 1.7, 7.7 and 36 mg/kg bw in males and 0, 1.84, 8.4 and 37.9 mg/kg bw in females) for 13 weeks. Ras in the positive-control group were treated with acrylamide and trimethyltin chloride. Concentration, homogeneity and stability of the test compound in the diet were confirmed. Mortality and clinical signs were monitored twice per day and the rats were given a detailed physical examination weekly. Body weight and food consumption were also determined weekly. The first ten rats from each group were given a battery of tests to assess neurological functions (including FOB and motor activity) approximately 1 week before exposure to profenofos and during weeks 3, 7, and 12. For necropsy, these rats were killed by whole-body perfusion and examined by a special histopathological investigation focusing on the brain, spinal and peripheral nerves, skeletal muscles and the eyes with the optic nerve. Further organs were examined if gross lesions occurred. Blood samples were taken from the other five rats of each group during weeks 3, 7, and 12 for determination of serum and erythrocyte acetylcholinesterase activity. At week 12, the whole brain was removed from these rats for determination of brain acetylcholinesterase activity. Inhibition of cholinesterase activity was calculated as a reduction relative to values for the appropriate control group. This study was conducted in accordance with GLP requirements and the US EPA FIFRA subdivision F guideline, addendum 10-neurotoxicity series 81, 82 and 83.

Dietary analysis indicated that the desired concentrations were achieved (range, 98–100% of nominal) and that the homogeneity was acceptable. The test compound was stable in the diet for up to 35 days when stored in closed containers at room temperature or in the refrigerator.

Overall body-weight gain was reduced at week 13 in the males and females at 600 ppm by about 7% and 11%, respectively. Reduced food consumption and reduced feed efficiencies were also observed at 600 ppm. At all dietary concentrations and at all time-points, a statistically significant inhibition of plasma and erythrocyte acetylcholinesterase activity was observed. There was a statistically significant inhibition of approximately 30% and 60% in plasma cholinesterase activity in male and female rats, respectively, in the group at 30 ppm and the inhibition increased to approximately 80% (males) and 93% (females) at the highest dose of 600 ppm. Erythrocyte acetylcholinesterase activity was also significantly inhibited by approximately 60% in male and female rats at 30 ppm and by approximately 78% at 135 ppm. Examination of the time-course of cholinesterase activity for rats at 30 and 135 ppm indicated that maximal inhibition was achieved after treatment for 3 weeks and that this level of inhibition was generally maintained throughout the study. At 600 ppm, there was a statistically significant inhibition of brain acetylcholinesterase activity of 12% in males and 20% in females at week 13. No clinical observations, ophthalmoscopic findings, FOB findings or motor activity effects related to profenofos were noted at any dietary concentration. Pathological examination revealed no evidence of compound-related neurotoxicity at any dose.

The NOAEL was 135 ppm, equal to 7.7 mg/kg bw per day, on the basis of statistically significant inhibition of brain acetylcholinesterase activity and reduced body weights, body-weight gains, reduced food consumption and reduced feed efficiency at 600 ppm, equal to 36.0 mg/kg bw per day. The report authors considered that depression of erythrocyte acetylcholinesterase activity at lower dietary concentrations was not an adverse effect but rather a sign of exposure, but the Meeting considered that this may simply have been a reflection of the relatively low sensitivity of their FOB end-point. Rats treated with the substances serving as positive controls gave the expected results (Pettersen & Morrissey, 1994b).

(c) Developmental neurotoxicity

In a preliminary study, groups of 15 time-mated Alpk:AP$_f$SD Wistar rats were given diets containing profenofos (purity 91.8%) at a concentration of 0, 4, 200, 400 or 600 ppm from day 7 of gestation until postnatal day 22. The achieved doses were 0, 0.3, 15.5, 30.2 and 46.1 mg/kg bw per

day during gestation and 0, 0.7, 33.9, 66.0 and 97.6 mg/kg bw per day during lactation and weaning. Day 1 of gestation was the day of confirmation of mating and postnatal day 1 was the day of littering. Rats were examined daily, clinical signs of toxicity and body weight were recorded on days 7, 8, 15 and 22 of gestation and on postnatal days 1, 5, 8, 12, 15 and 22. Food consumption was recorded at 3–4-day intervals during gestation and lactation. Concentration, homogeneity and stability of the test compound in the diet were confirmed. The sex, weight and clinical condition of each pup were recorded on postnatal days 1, 5, 8, 12, 15 and 22 . Plasma, erythrocyte and brain acetylcholinesterase activity was determined in dams on day 22 of gestation and postnatal day 22. Cholinesterase activity was also determined in pups on day 22 of gestation and postnatal days 5, 12 and 22. Staements of compliance with GLP and QA statement were provided, but as this was a preliminary study compliance with a regulatory guideline was not applicable.

Profenofos was stable in the diet at room temperature for 13 days and in the freezer for 28 days. The test compound was homogenously distributed in the diet. The mean concentrations were within 8% of nominal concentrations.

During gestation, body weight was slightly reduced (6%) in rats at 600 ppm and food consumption was reduced in rats at 400 or 600 ppm. On postnatal day 22, decreases in male (13%) and female pup weights (9%) and total litter weights (13%) were evident in the group at 600 ppm compared with values for controls. There were no other effects on litter or pup parameters.

In the parental rats, there were statistically significant reductions in plasma and erythrocyte acetylcholinesterase activity in rats at 200, 400 and 600 ppm at day 22 of gestation and postnatal day 22 . The inhibition was not dose-dependent and ranged from 76–86% for plasma cholinesterase and 42–53% for erythrocyte acetylcholinesterase activity. Brain acetylcholinesterase activity was significantly inhibited in the groups at 200, 400 and 600 ppm on postnatal day 22. The inhibition appeared to be dose-dependent and ranged from 21% at 200 ppm to 52% at 600 ppm. On day 22 of gestation, there was an 18% and 17% inhibition of brain acetylcholinesterase activity in rats at 400 and 600 ppm, respectively, but the reduction was not statistically significant. In pups there were no statistically significant effects on plasma, erythrocyte or brain cholinesterase activity on day 22 of gestation. A statistically significant inhibition of erythrocyte and brain acetylcholinesterase activity was only found on postnatal day 22, but there was no clear dose–response relationship. Brain acetylcholinesterase activity was inhibited by 25% in females in the group at 400 ppm and by 16% in males in the group at 600 ppm; erythrocyte acetylcholinesterase activity was inhibited by 33–46% in pups in the groups at 400 and 600 ppm. Inhibition of plasma cholinesterase activity increased during weaning and there was a 36–65% inhibition in pups exposed to profenofos at 200–400 ppm by postnatal day 22.

The NOAEL for parental toxicity was 4 ppm (0.3 mg/kg bw per day) on the basis of significant inhibition of brain acetylcholinesterase activity at doses of 200 ppm (15.5 mg/kg bw per day) and greater, and reduced body-weight gain and food consumption at 400 and 600 ppm. The NOAEL for offspring toxicity was 200 ppm (33.9 mg/kg bw per day) on the basis of reductions in brain acetylcholinesterase activity at doses of 400 ppm (66.0 mg/kg bw per day) and greater (Milburn, 2002).

In the main study of developmental neurotoxicity, groups of 30 mated Alpk:APfSD Wistar-derived rats were fed diets containing profenofos (purity, 91.8%) at a concentration of 0, 3, 60 or 600 ppm from day 7 of gestation to postnatal day 29. These dietary concentrations corresponded to daily doses of 0, 0.3, 5.1 and 50.6 mg/kg bw per day during gestation and 0, 0.5, 10.7 and 103.4 mg/kg bw per day during lactation and weaning. Concentration, stability and homogeneity of the test compound in the diet were determined. Rats were examined daily; clinical signs of toxicity, body weight and food consumption were recorded at intervals during gestation and up to termination on postnatal day 29. The parental animals were assessed in a FOB on days 10 and 17 of gestation and on postnatal days 2 and 9. The sex, weight and clinical condition of each pup were recorded on postnatal days 1 and 5. Where possible, litters were culled on postnatal day 5 to eight pups per litter with sexes

represented as equally as possible. Pups were selected on postnatal day 5 for the F1 generation; they were separated on day 29 and allowed to grow to adulthood. Rats in the F1 generation were examined daily, clinical examinations, food consumption and body weights were measured at intervals, and evaluations of motor activity, auditory startle response and assessments of learning and memory were also carried out. Rats were killed on postnatal day 63 and tissues were removed for neuropathological investigations. Additional satellite groups (five dams per group, five male and five female fetuses per group and five male and five female pups per group) were used to determine plasma, erythrocyte and brain acetylcholinesterase activity in dams on day 22 of gestation and postnatal day 22, in fetuses on day 22 of gestation and in pups on postnatal days 5, 12 and 22. The study was conducted in accordance with GLP regulations and the protocol complied with US EPA OPPTS guideline No 870.6300.

The results of dietary analyses indicated that the test compound was stable in the diet for 27 days when stored at room temperature or at nominally − 20 °C. The mean concentration and homogeneity of the test compound in the diet were within limits of acceptability (± 9% and 3% of nominal, respectively).

Body weights were slightly reduced in the parent females during late gestation (15%) and early in the postnatal period (5%) in rats at 600 ppm. Food consumption was also reduced during late gestation and during late lactation in rats of this group. There were no treatment-related effects on clinical observations, FOB measurements, reproductive parameters, litter losses or pup survival. On day 22 of gestation and on postnatal day 22 there was a statistically significant reduction in plasma cholinesterase activity of approximately 60% and 80% in the parental rats receiving profenofos at 60 and 600 ppm, respectively. There was a similar reduction, of approximately 55% of values for controls, in erythrocyte acetylcholinesterase activity in these groups at both time-points. At 600 ppm, brain acetylcholinesterase activity was decreased by 44% on day 22 of gestation, and by 26% (not statistically significant) on postnatal day 22.

Pup and total litter weights were reduced on postnatal day 5 in the group at 600 ppm and group mean body weights for F_1 rats in this group were lower than those of the controls from day 5 to day 29 in males and until day 36 in females. In the F_1 rats, there were no treatment-related effects on motor activity, auditory startle response, learning and memory or neurohistopathology. No statistically significant effects were noted on cholinesterase activity in pups in the group at 3 ppm. In the group at 60 ppm, only plasma cholinesterase activity was significantly inhibited (23%) at postnatal day 22. In males and females in the group at 600 ppm, plasma cholinesterase activity was significantly inhibited by up to approximately 50% at postnatal day 22 and erythrocyte cholinesterase activity was inhibited by up to 40% at the same sampling time. There was a statistically significant difference in brain acetylcholinesterase activity in female pups at day 5 (11% lower) but not at later sacrifice times. On postnatal day 12, absolute brain weight was decreased (by 4%) in the males at the highest dose only. The differences were no longer evident when the weights were adjusted to lower body weights of these animals. No treatment-related gross or microscopic pathological findings were noted in any treated group. Significant differences in various morphometric measurements were seen in males and females at the highest dose on postnatal days 12 and 63.

The NOAEL for maternal toxicity was 60 ppm, equal to 5.1 mg/kg bw per day, on the basis of inhibition of brain acetylcholinesterase activity on day 22 of gestation and day 22 of lactation, and reductions in body weight and food consumption at 600 ppm, equal to 50.6 mg/kg bw per day. The NOAEL for developmental neurotoxicity was 600 ppm, equal to 50.6 mg/kg bw per day. The NOAEL for offspring toxicity was 60 ppm, equal to 5.1 mg/kg bw per day, on the basis of inhibition of brain acetylcholinesterase activity, reduced body weight, decreased brain weights in males on postnatal day 12 and changes in the brain morphometric parameters at 600 ppm, equal to 50.6 mg/kg bw per day (Milburn, 2003).

(d) Delayed neurotoxicity

A first study was conducted with three groups of two male and two female White Leghorn chickens given profenofos (purity undefined) at a dose of 21.7, 46.4 or 60 mg/kg bw in polyethylene glycol as vehicle. Surviving chickens (only in the groups at 21.7 and 46.4 mg/kg bw) were given a second dose 21 days later. For protection against the acute toxic effects of profenofos, atropine was administered intra-

muscularly 60 min before the second dose. Tri-orthocresyl phosphate was given to chickens in a positive-control group. Clinical signs of toxicity and mortality were recorded up to 42 days after the second dose. At the end of the observation period, the remaining birds were killed and samples of muscle and spinal cord were taken for histopathology. The study was not conducted in accordance with GLP requirements, but the procedure used was as recommended by the United Kingdom Ministry of Agriculture, Fisheries and Food (MAFF), Pesticide Safety Precaution Scheme, working document No 2, appendix B, 1967.

All the chickens at 60 mg/kg bw died after the first dose and only those in the group at the lowest dose survived the two treatments. Clinical signs of toxicity were salivation, asynchronisms of the extremities, curved position, apathy and ruffled feathers. Neither symptoms of delayed neurotoxicity nor histological changes in spinal cord or peripheral nerve could be detected. The birds in the concurrent positive-control groups treated with tri-orthocresyl phosphate showed the expected reactions (ataxia, deterioration of the reflexes, swelling, fragmentation and disruption of myelin sheaths) by day 18 after treatment and the histopathology findings were also as expected (Krinke et al., 1974).

In a second study, chickens of the White Leghorn strain (sex not specified) were given profenofos (purity, 89.5%) at a dose of 30 or 45.7 mg/kg bw. The doses were based on an initial assessment of the acute toxicity of profenofos, which showed the LD_{50} to be 45.7 mg/kg bw. Surviving birds were given a second dose 21 days after the initial dose, but owing to the unexpectedly high mortality after the first dose, the second was reduced to 17.1 mg/kg bw, which was the revised LD_{50} on the basis of mortality after the first dose. Birds in a positive-control group were given tri-orthocresyl phosphate. The birds were observed daily for mortality and possible neurotoxic signs for up to 21 days after the second dose. Body weight was measured on days 0, 21 and 42 and food consumption was measured weekly. All birds that died during the study were given a gross necropsy. At the end of the study, the sciatic nerves, brain and spinal cord were examined in surviving birds. The results of this study had not been independently validated.

Forty-one of the 50 birds at 45.7 mg/kg bw and 28 of the 40 birds at 30 mg/kg bw died within 21 days of dosing, mostly within 24 h. There was only one further death when surviving birds of both groups were given the second dose 21 days after the first, even without pre-treatment with atropine. Clinical signs noted after the first dose were lethargy and salivation. Slight lethargy was also apparent for 24–48 h after the second dose. There were no behavioural signs of delayed neurotoxicity. In particular, no signs of locomotor disturbances were observed. Reduced body-weight gains and food consumption were apparent in treated birds, particularly after the first dose. Gross and histopathological studies of neural tissues from these birds did not reveal any treatment-related findings. The concurrent positive-control group showed the expected established signs of neurotoxicity by day 8 of study (Reinart, 1978).

These studies demonstrate that profenofos caused no delayed neurotoxic effects in adult chickens even at very high (sublethal) doses.

(e) Antagonistic agents

A protective effect of atropine given early after the oral administration of profenofos in rats or intraperitoneal administration in chicks and mice was demonstrated by a reduction in mortality and signs of toxicity (e.g. salivation, tremors, sedation, and convulsions) typical of exposure to anticholinesterase. The effect of oximes was limited, probably due to rapid ageing (Sachsse & Bathe, 1976; Gfeller & Kobel, 1984; Glickman et al., 1984). These investigations were not conducted to GLP or to any regulatory guideline.

(f) Potentiation studies

Potentiation studies were divided into two phases. First, the oral LD_{50} for each compound was experimentally determined in rats. In the second phase, the LD_{50} values for the equitoxic mixtures of the insecticides were evaluated and compared with the theoretical LD_{50} values derived from an assumption of strictly additive toxicity. The procedure for the determination of the acute oral LD_{50} for each com-

pound and the equitoxic mixtures was essentially the same. The compounds were suspended in aqueous carboxymethyl cellulose and given to groups of five male and five female Tif-RAIf (SPF) rats that were then observed for 14 days. None of the studies were conducted to GLP or to any regulatory guideline.

No potentiation effects were found when mixtures of profenofos with methidathion (GS 13'005), methacrifos (CGA 20'168) or diazinon (G 24'480) were given to rats in equitoxic doses (Sachsse & Bathe, 1977, Sachsse & Bathe, 1978). In contrast to this, a potentiation experiment with profenofos Q (active ingredient of higher purity; from the toxicological point of view regarded as equivalent to CGA 15'324) and malathion showed a strong potentiation of the acute oral toxicity.

The experimental LD_{50} values of the single compounds were 377 (range, 282–545) mg/kg bw for profenofos (purity, 96.3%) and 4658 (3320–8504) mg/kg bw for malathion (purity not stated). The equitoxic mixture led to an experimental LD_{50} of 76 (range, 45–125) mg/kg bw, while the theoretical (calculated) LD_{50} was 2517 mg/kg bw (Sarasin, 1981).

Profenofos (purity, > 95%) at doses of 0.5 to 5.0 mg/kg bw given intraperitoneally to mice strongly inhibited the liver microsomal esterase(s) responsible for the hydrolysis of *trans*-permethrin. At an intraperitoneal dose of 25 mg/kg bw, profenofos increased the toxicity of fenvalerate by more than 25-fold, and that of malathion by more than 100-fold. This potentiation did not occur with *trans*-permethrin (Gaughan et al., 1980).

3. Studies on metabolites

No studies have been conducted with metabolites of profenofos. However, studies conducted in 1981 with analogous alkyl phosphate metabolites in vitro demonstrated a complete lack of anticholinesterase activity (Chukwudebe et al., 1984).

4. Observations in humans

Workers engaged in the manufacture of profenofos were given a medical examination annually between 1980 and 1998. No health effects related to exposure to profenofos were identified (Novartis Crop Protection AG Assessment, 1998).

In a biological monitoring study, cotton was sprayed with different formulations of profenofos, using manual equipment. Six workers in Multan, Central Pakistan, were monitored daily during a 4-day spraying campaign. Cholinesterase activities were determined in whole blood and were found to be slightly below the values determined before exposure. Individual cholinesterase activities declined from 100% (pre-test) to an average of 81%, with a lowest value of 73%. None exceeded the threshold of 30% inhibition that was considered to be biologically significant. The handling of the active ingredient and of its formulations is subject to the usual precautionary measures recommended for the handling of insecticidal organophosphates (Loosli, 1989).

There are seven reports of effects after exposure to profenofos during application of the product. In one, considered of moderate severity, the operator used only marginally protective equipment. He was given atropine and fully recovered. The other six reports described adverse incidents that were of minor severity and also involved people using the product without protective equipment or with marginally protective equipment. The symptoms were transient and resolved spontaneously. The exposed persons fully recovered.

Finally, one accidental exposure had been notified. This involved a child (bystander) who inhaled spray mist. Again the symptoms were transient, the effects disappeared without the need for treatment and the child fully recovered.

Comments

Biochemical aspects

[Phenyl-^{14}C]profenofos was rapidly absorbed and eliminated after oral administration to rats. Total radioactivity eliminated via the urine and faeces exceeded 99% of the administered dose for a single dose of 1 or 100 mg/kg bw by gavage and repeated doses of 1 mg/kg bw by gavage. Elimination was rapid, with about 95% of the total radiolabel being excreted in the urine within the first 24 h in all treated groups. For all doses, less than 4% of the radiolabel was excreted in the faeces. The concentration of radiolabel in tissues and organs reached a maximum after 2 h and remained at similar levels until 8 h after dosing. By 72 h, the tissue concentration of radiolabel was minimal. The absorption, distribution and excretion of 14C-labelled profenofos was not sex- or dose-dependent in the range of 1 to 100 mg/kg bw and was unaffected by pre-treatment with unlabelled profenofos for 14 days. Unchanged profenofos was detected in the faeces, but the amount was very small (approximately 1–2% of the administered dose), and this was probably the proportion of the dose that was not absorbed. Four major metabolites were present in urine and no unchanged profenofos was detected. The major metabolites were the sulfate and glucuronide conjugates of 4-bromo-2-chlorophenol that were formed by hydrolysis of the aryloxy–phosphorus bond followed by conjugation with sulfate or glucuronic acid. The other two metabolites were formed by cleavage of the phosphorus–sulfur bond either by loss of the propyl group or hydrolysis. The 4-bromo-2-chloro-phenol was detected in some urine samples, but probably arose as a result of hydrolysis of the conjugates after excretion.

Toxicological data

The oral LD_{50} for profenofos ranged from 358 to 1178 mg/kg bw in rats. The oral LD_{50} for profenofos was 298 mg/kg bw in mice and 700 mg/kg bw in rabbits. The clinical signs detected in all the studies of acute toxicity were typical of cholinergic poisoning, which appeared at doses greater than 100 mg/kg bw. Profenofos was of low toxicity when administered by the dermal route to rats (LD_{50}s, > 2000 and 3300 mg/kg bw). More varied results were obtained after dermal application to rabbits, with LD_{50}s ranging from 131 to 2560 mg/kg bw depending on method of application (semi-occlusive, abraded skin or massaging). Profenofos was of low toxicity on exposure by inhalation, the LC_{50} being > 3.36 mg/l air. Profenofos was moderately irritating to skin and mildly irritating to the eye and was shown to be a sensitizer under the conditions of the Magnusson & Kligman test and in the local lymph-node assay.

The primary effect of profenofos in studies of acute toxicity and short- and long-term studies of toxicity was inhibition of acetylcholinesterase activity and this was associated with signs of neurotoxicity at high levels of inhibition. Profenofos is a racemic mixture of the two optical isomers at the chiral phosphorus atom. The S (−) isomer is a markedly more potent inhibitor of acetylcholinesterase in vitro than the R (+) isomer. The inhibited acetylcholinesterase ages rapidly, an effect that prevents spontaneous reactivation. Rapid ageing would lead to a cumulative inhibitory effect after repeated exposures to profenofos, and would also render reactivation therapy with oximes ineffective.

In a short-term repeat-dose study, no clinical signs of toxicity were observed in rats given diet containing profenofos at a concentration of 1000 ppm, equal to 85 mg/kg bw per day, for 8 weeks. Reduced food intake and body-weight gain were apparent at this dose and also at a dose of 100 ppm, equal to 8.4 mg/kg bw per day, which was given for 13 weeks. Inhibition of cholinesterase activity was the only other effect noted. Erythrocyte cholinesterase activity was inhibited by more than 20% at doses of 30 ppm, equal to 2.4 mg/kg bw per day, and greater. Brain acetylcholinesterase activity was inhibited at 1000 ppm, equal to 85 mg/kg bw per day. The NOAEL for inhibition of brain acetylcholinesterase activity was 300 ppm, equal to 22.0 mg/kg bw per day.

Inhibition of brain acetylcholinesterase activity and clinical signs consistent with neurotoxicity were observed in rats exposed to profenofos at a concentration of 0.07 mg/l per day by inhalation for 21 days.

In three studies of dermal toxicity in rabbits, the overall NOAEL for inhibition of brain acetylcholinesterase was 2.5 mg/kg bw per day on the basis of significantly reduced activity at 5 mg/kg bw per day.

Three studies were carried out in dogs given profenofos orally for 90 days, 6 months, or 1 year. Profenofos was given in the diet in the 90-day and 6-month studies, and daily in gelatin capsules in the 1-year study. No clinical signs of toxicity were recorded in these studies, the 6-month and 1-year studies including neurological examinations (NOAEL for clinical signs, 12.5 mg/kg bw per day). Brain acetylcholinesterase activity was significantly inhibited in males at 5 mg/kg bw per day in the 90-day study, but not in either sex at 2.9 or 14.4 mg/kg bw per day in the 6-month study, or at 1 or 12.5 mg/kg bw per day (the highest dose tested) in the 1-year study. Hence, for brain acetylcholinesterase inhibition, the overall NOAEL in these three studies in dogs was 2.9 mg/kg bw per day. Haematology parameters (erythrocyte count, haemoglobin concentration and erythrocyte volume fraction) were reduced; however, they were not considered to be toxicologically significant since there was no clear dose–response relationship, and the small changes observed were within the range for historical controls. Treatment of dogs with profenofos at 12.5 mg/kg bw per day for 1 year was also associated with an increase in binucleated perilobular hepatocytes, bile-duct hyperplasia and an increase in bile pigments in kidney tubules. These pathological findings were minimal in severity and were not observed in the 90-day or 6-month studies of toxicity.

Profenofos was not mutagenic in an adequate battery of studies of genotoxicity.

The Meeting concluded that profenofos is unlikely to be genotoxic.

In long-term studies, treatment of mice and rats with profenofos did not adversely affect survival; there were no clinical signs of toxicity, no increase in the incidence of tumour formation and no treatment-related changes in either gross pathology or histopathology. Plasma and erythrocyte cholinesterase activity were significantly reduced in mice given diet containing profenofos at 30 ppm, equal to 4.5 mg/kg bw per day, and in rats at 100 ppm, equal to 5.7 mg/kg bw per day. In female mice, there was a statistically significant inhibition of brain acetylcholinesterase activity (25%) at termination of the group at 100 ppm, equal to 14.2 mg/kg bw per day, resulting in a NOAEL of 30 ppm, equal to 4.5 mg/kg bw per day. The NOAEL in the 2-year study of carcinogenicity in rats was 100 ppm, equal to 5.7 mg/kg bw per day, the highest dose tested. Profenofos was not carcinogenic in mice and rats up to the highest dose tested. Although overt toxicity was not observed in the study in rats, the Meeting considered that the available database was sufficient to evaluate the carcinogenic potential of profenofos.

In view of the lack of genotoxicity, the absence of carcinogenicity in mice and rats, and any other indication of carcinogenic potential, the Meeting concluded that profenofos is unlikely to pose a carcinogenic risk to humans.

Multigeneration studies have shown that profenofos has no effect on reproduction at doses of up to 400 ppm, equivalent to 35 mg/kg bw per day. The NOAEL for parental and pup toxicity was 100 ppm, equivalent to 7.0 mg/kg bw per day, on the basis of reduced body-weight gains and food consumption at 400 ppm, equivalent to 35 mg/kg bw per day, and the NOAEL for reproductive toxicity was 400 ppm, the highest dose tested.

Profenofos did not cause developmental effects in rats or rabbits. Clinical signs typical of cholinesterase inhibition were noted in rabbits given profenofos at 175 mg/kg bw per day and approximately 50% of the animals died. There were no treatment-related effects on the mean number of implantations, litter size, fetal body weight or embryolethality and there were no significant increases in variations or malformations in the fetuses. The NOAEL for maternal toxicity was 30 mg/kg bw per day and the NOAEL for developmental toxicity was 175 mg/kg bw per day, the highest dose tested.

Studies of developmental toxicity in rats, maternal toxicity, which included clinical signs typical of cholinesterase inhibition, and deaths were observed at the highest dose of 120 mg/kg bw per day. There was no evidence for prenatal toxicity at either of these doses and the type and incidence of fetal malformations and variations was unaffected by treatment. The NOAEL for maternal toxicity was 90 mg/kg bw per day and the NOAEL for developmental toxicity was 120 mg/kg bw per day, the highest dose tested.

The Meeting concluded that profenofos is not teratogenic.

The potential for profenofos to cause developmental neurotoxicity had also been investigated in rats. In a preliminary range-finding study, rats were given diets containing profenofos at a concentration of 0, 4, 200, 400 or 600 ppm, equal to 0, 0.7, 33.9, 66.0 or 97.6 mg/kg bw per day. In this study, dose-dependent inhibition of the brain acetylcholinesterase activity was observed in dams at ≥ 200 ppm on postnatal day 22. The NOAEL for inhibition of brain acetylcholinesterase activity in dams was 4 ppm, equal to 0.7 mg/kg bw per day. A statistically significant inhibition of brain acetylcholinesterase activity of > 20% and 16% was found in female pups at ≥400 ppm and male pups at 600 ppm, respectively. In the main study of developmental neurotoxicity, rats were given diets containing profenofos at a concentration of 0, 3, 60 or 600 ppm (equal to 0, 0.3, 5.1 or 50.6 mg/kg bw per day). At 600 ppm in dams, brain acetylcholinesterase activity was decreased by 44% on day 22 of gestation, and by 26% (not statistically significant) on day 22 of lactation, and body weights and food consumption were reduced. A statistically significant inhibition of brain acetylcholinesterase activity was observed in female pups at 600 ppm compared with controls on day 5 (11% lower) but not at later times. At 600 ppm, there was a statistically significant reduction in pup body weights (11–12%). No effects on functional parameters or neurohistopathology were observed. The NOAEL for maternal toxicity was 60 ppm, equal to 5.1 mg/kg bw per day, on the basis of inhibition of brain acetylcholinesterase activity on day 22 of gestation and day 22 of lactation and reductions in body weight and food consumption at 600 ppm, equal to 50.6 mg/kg bw per day. The overall NOAEL for inhibition of brain acetylcholinesterase in pups was 60 ppm, equal to 5.1 mg/kg bw per day. The NOAEL for developmental neurotoxicity was 600 ppm, equal to 50.6 mg/kg bw per day, the highest dose tested.

In two studies of acute neurotoxicity in rats, there were reversible signs typical of poisoning with acetylcholinesterase inhibitors (diarrhoea, miosis, lacrimation, tremor), peaking 4 h after administration of profenfos at 380 mg/kg bw by gavage. Lesser effects were seen at 200 mg/kg bw (hypoactivity, soft faeces), and there were no effects in the FOB at 190 mg/kg bw (the NOAEL for clinical signs). There was significant inhibition of brain acetylcholinesterase activity (by 37% in males and 43% in females) at 4 h after dosing at 400 mg/kg bw, with a NOAEL of 100 mg/kg bw. Inhibition was absent after a recovery period of 14 days.

There were also no clinical signs of toxicity, and no adverse findings in a FOB or effects on motor activity in a 90-day study of neurotoxicity in rats. Pathological investigation revealed no evidence of treatment-related toxicity. At the highest dose of 600 ppm, equal to 36 mg/kg bw per day, there was a reduction of approximately 10% in body-weight gain. At 600 ppm, there was a statistically significant inhibition of brain acetylcholinesterase activity of 12% in males and 20% in females at week 13. The NOAEL for brain acetylcholinesterase inhibition was 135 ppm, equal to 7.7 mg/kg bw per day.

Profenofos did not induce delayed neuropathy in chickens given two doses at 45.7 mg/kg bw (maximum tolerated dose) and then at 17.1 mg/kg bw, separated by an interval of 21 days (atropine protection being given as soon as clinical signs appeared).

No cases of adverse effects have been reported among workers involved in the manufacture of profenofos. In a biological monitoring study, whole-blood cholinesterase activity was inhibited by less than 30% in six workers who were monitored daily for 4 days during spraying of profenofos.

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

Toxicological evaluation

Erythrocyte acetylcholinesterase activity was found to be significantly more sensitive to profenofos than was brain acetylcholinesterase activity in rats, mice, rabbits, and dogs. However, in no species were any signs of toxicity seen at doses that did not also produce significant inhibition of brain acetylcholinesterase. The Meeting thus concluded that inhibition of brain acetylcholinesterase activity was the more appropriate end-point for risk assessment of profenofos.

The Meeting established an ADI of 0–0.03 mg/kg bw per day based on an overall NOAEL of 2.9 mg/kg bw per day identified on the basis of inhibition of brain acetylcholinesterase activity in three short-term studies in dogs and using a safety factor of 100. This ADI was supported by the NOAEL of 5.1 mg/kg bw per day identified on inhibition of maternal and pup brain acetylcholinesterase activity in a study of developmental neurotoxicity in rats and a NOAEL of 4.5 mg/kg bw per day identified on the basis of inhibition of brain acetylcholinesterase activity in a 2-year study in mice.

The Meeting established an ARfD of 1 mg/kg bw based on a NOAEL of 100 mg/kg bw in studies of acute neurotoxicity in rats, identified on the basis of clinical signs of neurotoxicity seen at ≥ 200 mg/kg bw and inhibition of brain acetylcholinesterase activity at 400 mg/kg bw and using a safety factor of 100. The appropriate study for establishing the ARfD was the study of acute neurotoxicity since there was no evidence of developmental effects. This ARfD was considered to be protective against any clinical signs of acetylcholinesterase inhibition seen in studies of acute oral toxicity.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year studies of toxicity and carcinogenicity[a]	Toxicity	4.5 mg/kg bw per day	14.2 mg/kg bw per day
		Carcinogenicity	14.2 mg/kg bw per day[c]	—
Rat	Two-year studies of toxicity and carcinogenicity[a]	Toxicity	5.7 mg/kg bw per day[c]	—
		Carcinogenicity	5.7 mg/kg bw per day[c]	—
	Multigeneration study of reproductive toxicity[a]	Parental	7.0 mg/kg bw per day	35.0 mg/kg bw per day
		Reproductive toxicity	35.0 mg/kg bw per day[c]	—
		Offspring toxicity	7.0 mg/kg bw per day	35.0 mg/kg bw per day
	Developmental toxicity[b]	Maternal toxicity	90.0 mg/kg bw per day	120.0 mg/kg bw per day
		Embryo/fetotoxicity	120.0 mg/kg bw per day[c]	—
	Developmental neurotoxicity[a]	Parental toxicity	5.1 mg/kg bw per day	50.6 mg/kg bw per day
		Offspring toxicity	5.1 mg/kg bw per day	50.6 mg/kg bw per day
	Acute neurotoxicity[b,d]	Toxicity	100.0 mg/kg bw	400.0 mg/kg bw per day
Rabbit	Developmental toxicity[b]	Maternal toxicity	30.0 mg/kg bw per day	60.0 mg/kg bw per day
		Embryo/fetotoxicity	175.0 mg/kg bw per day[C]	—
Dog	Studies of toxicity[d]	Toxicity	2.9 mg/kg bw per day	12.5 mg/kg bw per day

[a] Dietary administration.
[b] Gavage administration.
[c] Highest dose tested.
[d] The results of two or more studies were combined.

Estimate of acceptable daily intake for humans

0–0.03 mg/kg bw

Estimate of acute reference dose

1 mg/kg bw

Information that would be useful for the continued evaluation of the compound

Results from epidemiological, occupational health and other such observational studies of human exposures

Critical end-points for setting guidance values for exposure to profenofos

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	About 94% within 24 h
Dermal absorption	Approximately 90%
Distribution	Widely distributed
Potential for accumulation	Low, no evidence of accumulation
Rate and extent of excretion	94% in urine within 24 h
Metabolism in animals	> 95% by conversion of the phosphorothiolate group to a variety of hydrolysis products
Toxicologically significant compounds in animals, plants and the environment	Parent
Acute toxicity	
Rat, LD_{50}, oral	358–1178 mg/kg bw
Rat, LD_{50}, dermal	3300 mg/kg bw
Rat, LC_{50}, inhalation	3.36 mg/l
Skin irritation	Moderately irritating
Eye irritation	Mildly irritating
Guinea-pig, skin sensitization (test method used)	Sensitizer (Magnusson & Kligman and local lymph-node assay)
Short-term studies of toxicity	
Target/critical effect	Inhibition of brain acetylcholinesterase activity
Lowest relevant oral NOAEL	2.9 mg/kg bw per day (dogs)
Lowest relevant dermal NOAEL	2.5 mg/kg bw per day
Lowest relevant inhalation NOAEC	< 0.07 mg/l air
Genotoxicity	
	No genotoxic potential
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Inhibition of brain acetylcholinesterase activity
Lowest relevant NOAEL	4.5 mg/kg bw per day (2-year study in mice)
Carcinogenicity	Not carcinogenic
Reproductive toxicity	
Reproduction target/critical effect	No reproductive effects
Lowest relevant reproductive NOAEL	400 ppm (35 mg/kg bw per day) (rats)
Developmental target/critical effect	No developmental effects
Lowest relevant developmental NOAEL	120 mg/kg bw per day (rats)

Neurotoxicity/delayed neurotoxicity	
Acute neurotoxicity	Inhibition of brain acetylcholinesterase activity, NOAEL was 100 mg/kg bw per day (rats)
Developmental neurotoxicity	Inhibition of brain acetylcholinesterase activity, NOAEL was 5.1 mg/kg bw per day (rats)
Delayed neuropathy	No delayed neurotoxicity, NOAEL was 45.7 mg/kg bw (chickens)
Medical data	
	No detrimental effects on agricultural workers

Summary

	Value	Study	Safety factor
ADI	0–0.03 mg/kg bw	Dog, studies of oral toxicity	100
ARfD	1 mg/kg bw	Rat, study of acute neurotoxicity	100

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