

PIRIMIPHOS-METHYL

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EXPLANATION

Pirimiphos-methyl was previously evaluated by JMPR in 1974 and 1976 (Annex 1, references 22 and 26). An ADI of 0-0.01 mg/kg bw was established in 1976. Relevant data from the previous monograph and monograph addendum are incorporated into the present monograph.

EVALUATION FOR ACCEPTABLE DAILY INTAKE**Biochemical aspects****Absorption, distribution, and excretion****Rats**

Oral administration of 0.6 mg 2-¹⁴C-ring labelled pirimiphos-methyl/kg bw to 5 male rats resulted in a mean urinary excretion of 80.7% and mean faecal excretion of 7.3% in 24 h, indicating rapid absorption. At 96 h, 86.0% and 15.2% of the administered dose had been excreted in urine and faeces, respectively. Nine metabolites (unidentified) were present in the urine (Bratt & Dudley, 1970).

Female rats given 7.5 mg 2-¹⁴C pirimiphos-methyl/kg bw orally were bled (cardiac puncture, 3 rats per time interval) at 0.5, 1, 3, 5, 7 or 24 h post-dosing. Maximum blood levels (at 0.5 h) were 2-3 µg/ml, declining by 50% 1 h post-dosing. By 24 h, levels of ¹⁴C in blood were 0.2 to 0.3 µg/ml, and of pirimiphos-methyl, 0.01-0.02 µg/ml. Rats treated for 4 days at 7.5 mg 2-¹⁴C pirimiphos-methyl/kg bw/day and sacrificed at 24 h intervals did not show any increase in blood levels with time. Tissue levels of total radioactivity in liver, kidney and fat over the 4 day period were generally below 2 mg pirimiphos-methyl equivalents/kg tissue (levels of unchanged pirimiphos-methyl being less than 0.15 mg/kg tissue). There was no evidence of tissue accumulation (Mills, 1976).

Adult male Wistar rats were intubated with 1 mg ¹⁴C pirimiphos-methyl/kg bw/day. Four groups of 3 animals were dosed for 3, 7, 14 or 21 days and sacrificed 24 h after the final dose. A further five groups of 3 rats were similarly dosed for 28 days and sacrificed 1, 3, 7, 14, or 28 days post-dosing. Each of the 9 groups had one rat, undosed, as a control. Following sacrifice, samples of liver,

kidney, muscle, fat, erythrocytes and plasma were taken for analyses. Urine and faeces were collected from 2 rats over a 24 h period following the seventh dose. Recovery of ^{14}C from ^{14}C pirimiphos-methyl added to control tissues was $96.9 \pm 5.2\%$. In all tissue samples at all time intervals, concentration of radioactivity was very low, close to or below detection limits. Levels did not increase with repeated dosing. Liver concentrations were fairly constant (0.03 ppm) and in some kidney samples, similar levels were detected. In other tissues, radioactivity concentration was generally below the limits of detection (0.04-0.06 ppm). Three days after cessation of dosing one animal had detectable levels of kidney radioactivity concentrations. At 7 days and subsequent days, no residues were found. Excretion was between 70 and 80% of a single dose, following administration of 7 consecutive doses, providing evidence of rapid metabolism and elimination rather than poor absorption (Hawkins & Moore, 1979).

Dogs

Male beagle dogs (1/dose level) given 18.4 or 16.7 mg 2- ^{14}C -ring labelled pirimiphos-methyl/kg bw by capsule excreted 64.4% or 82.5% of the administered dose in the urine, and 17.3% or 13.3% in the faeces, respectively, in 48 h. Nine metabolites (unidentified) were present in urine (Bratt & Dudley, 1970).

Goats

In the lactating goat, following a single dose of 0.12 mg 2- ^{14}C -ring labelled pirimiphos-methyl/kg bw by capsule, 87% and 4% were excreted in urine and faeces, respectively, over 8 days. 80.6% and 2.7% were excreted in urine and faeces, respectively, during the final 48 h. Milk residues during the first 24 h post-dosing indicated a total residue of 0.035 ppm, of which only 0.003 ppm was parent compound. After 24 h residues declined to 0.004 ppm or less (Bowker *et al.*, 1973).

A lactating female goat was orally dosed by gelatin capsule, twice daily, with 40 mg ^{14}C -pirimiphos-methyl ($7.1 \times 10^6\text{Bq}$) labelled in the 2 position of the pyrimidine ring, for 7 consecutive days (equivalent to 45 ppm in the diet). Chemical and radiochemical purity exceeded 99%. At sacrifice, 78.1 and 11.3% of total administered radioactivity had been excreted in urine and faeces, respectively. Residues in milk were approximately 0.2% of the total administered dose, the highest residue being 0.208 ppm pirimiphos-methyl equivalents at day 2 of dosing. Levels plateaued by day 4 at about 0.15 ppm. Sixteen hours after sacrifice, radioactive residues in liver, kidney, muscle and fat were 0.31, 0.5, 0.44, and 0.067 ppm, respectively. The major components in fat were unchanged pirimiphos-methyl (55.2% of total radioactive residue) and 0-(2-ethylamino-6-methylpyrimidine-4-ol)0,0-dimethyl phosphorothioate (17.1%), and those in the remaining tissues and in milk were 2-diethylamino-6-methylpyrimidine-4-ol, 2-ethylamino-6-methylpyrimidine-4-ol, and 2-amino-6-methylpyrimidine-4-ol (Skidmore *et al.*, 1985).

Cows

In the cow following a single oral dose of 0.5 mg 2-¹⁴C-labelled pirimiphos-methyl/kg bw, excretion was similar to that in goat, 85% and 14% of the administered dose being excreted in urine and faeces, respectively, over 7 days. During the first 3 days post-dosing, 0.35% of the labelled was excreted in the milk (residue level, 0.04 ppm of which less than 2% was unchanged pirimiphos-methyl and phosphorus-containing metabolites). Hydroxypyrimidine hydrolyses products or their conjugates constituted the major portion of the milk residues (Bullock *et al.*, 1974).

Hens

Hens given a single oral dose of 2, 9 or 20 mg-¹⁴C-labelled pirimiphos-methyl excreted over 70% of the administered dose within 24 h. Three major metabolites, namely parent pyrimidine (4.7% of dose), 2-ethyl-amino-4-hydroxy-6-methyl pyrimidine (25% of dose) and 2-amino-4-hydroxy-6-methyl pyrimidine (31%) were identified. When fed for 289 days with dietary concentrations of 4 ppm, levels of parent compound in white and yolk of eggs never exceed 0.001 ppm although total ¹⁴C levels increased to 0.032 to 0.038 ppm over 16 days, and then fell (to equilibrium) to 0.026 to 0.028 ppm, 86-90% of which comprised water soluble metabolites. Hens fed dietary concentrations of 32 ppm for 7 days yielded eggs containing 0.007 (whites on day 3) and 0.012 (yolks on day 6) ppm pirimiphos-methyl. "Total" pirimiphos-methyl plus equivalents ranged from 0.08 to 0.15 ppm in whites plus yolk. In muscle, residues of 0.31 and 0.16 ppm were present following exposure to 32 and 4 ppm dietary concentrations, respectively (Green *et al.*, 1973).

Two hens were dosed orally by gelatin capsule with a mean dose of 1.52 mg ¹⁴C-pirimiphos-methyl (60.75 μ Ci)/hen for 14 consecutive days. Radiochemical purity was > 95%. Residues of ¹⁴C in breast muscle were 0.59% and 0.39 ppm. No unchanged pirimiphos-methyl was detected. The majority of the residue (74%) was identified as free and conjugated 2-amino-6-methyl-pyrimidin-4-ol. Radioactive residues in yolk appeared to plateau about days 7-10, but residues in albumen were inconsistent. At least 7 different compounds were identified as being present in the eggs, the major ones being 2-ethylamino-6-methyl-pyrimidine-4 ol and 2-amino-6-methyl-pyrimidine-ol (Hall *et al.*, 1979).

¹⁴C-Pirimiphos-methyl, labelled at the 2 position of the pyrimidine ring was administered to 3 hens by gelatin capsule, twice daily for 14 days. The mean dose/day was 2.54 mg pirimiphos-methyl (98.4% purity), equivalent to 50 ppm in the diet. An additional bird was fed capsules treated similarly to those fed to experimental birds, but containing no pirimiphos-methyl. Eggs were collected daily and excreta at 24 h intervals. Sixteen hours after the final dose, hens were killed, and tissues were taken for analysis. A mean value of 97.5% of the administered dose was excreted over the 14 day period. At sacrifice, liver, peritoneal fat,

subcutaneous fat, leg muscle and breast muscle contained 0.2, 0.093, 0.11, 0.67 and 1.3 ppm pirimiphos-methyl equivalents, respectively. In fat, approximately 72% of the radioactive residue was unchanged pirimiphos-methyl, which was only found elsewhere (9.5% of the total radioactive residue) in egg yolk. In muscle tissue the major residue was 2-amino-6-methylpyrimidin-4-ol and 2-ethylamino-6-methylpyrimidine-4-ol. These compounds were also found in liver in both free and conjugated forms (Skidmore & Tegala, 1985).

Biotransformation

The metabolism of pirimiphos-methyl in Wistar rats (5 males) given 100 mg ¹⁴C-labelled pirimiphos methyl/kg bw and in one beagle dog given 20 mg ¹⁴C-labelled pirimiphos-methyl/kg bw was investigated by TLC separation of urinary metabolites. Twelve metabolites were detected in rat, and 11 in dog urine. Five of the metabolites were identified. In both species, 2-ethylamino-4-hydroxy-6-methyl pyrimidine was the major urinary metabolite (30% of dose). The next most predominant metabolite in dog was 4-O(2-diethylamino-6-methylpyrimidinyl)-β-D-glucosiduronic acid (11% of dose) and in the rat, an unidentified phosphorus-containing product thought to be a dealkylated derivative of either pirimiphos-methyl or its oxygen analogue (12% of dose). Other identified metabolites were 2-amino-4-hydroxy-6-methyl pyrimidine (8% and 5% of dose in rat and dog, respectively) (Green *et al.*, 1973; Bratt & Jones, 1973).

These studies indicate that the P-O-C bond of pirimiphos-methyl is extensively cleaved and that N-de-ethylation and/or conjugation are further steps in the metabolism of the pyrimidine leaving group. Although the oxygen analogue of pirimiphos-methyl was not detected as a urinary metabolite, the fact that cholinesterase inhibition occurs *in vivo* suggests that the oxygen analogue is also formed and may be an intermediate step leading to the identified urinary products (Annex 1, reference 23).

Effects on enzymes and other biochemical parameters

The only biochemical effects consistently noted in acute or chronic toxicity tests was inhibition of cholinesterase. A group of 36 male rats were given single oral doses of 1450 mg pirimiphos-methyl/kg bw. Symptoms were noted and they were sacrificed at intervals up to 4 days after dosing for measurements of brain, plasma and red cell cholinesterase activity. Few signs were noted at 6 h after dosing when cholinesterase inhibition was 0, 35 and 51%, respectively, for brain, red cell and plasma. Clear signs of poisoning only became apparent by 24 h when brain was inhibited by 46% and red cell and plasma by 70 and 80%, respectively. Recovery of cholinesterase activity began to be apparent by 72 h. Plasma cholinesterase activity had completely recovered by 96 h but red cell and brain remained 47 and 30% inhibited, respectively, at this time (Clark, 1970). From these studies it appears that inhibition of brain cholinesterase by 40% or more results in obvious signs of toxicity (Annex 1, reference 23).

A group of 25 male (probably Wistar CFT, but not specified) were dosed with 1000 mg/kg bw. Twenty-five additional rats served as controls. Five rats/group were sacrificed at 4, 8, 24, 48 or 72 h post-dosing and plasma and brain cholinesterase and non-specific carboxylesterase activities were measured. Plasma cholinesterase inhibition was rapid (60% inhibition by 4 h) whereas brain cholinesterase inhibition was slower (36% by 8 h). Both attained maximum inhibition by 24 h (93% for plasma and 61% for brain). Recovery was apparent at 48-72 h in both enzymes, but that for brain was slower. Non-specific esterase (NSE) activity was inhibited, attaining maximum inhibition (plasma 80%, and brain 47%) at 24 h. Inhibition of NSE was less than for cholinesterase, and recovery in plasma was more rapid. In brain, NSE and cholinesterase activity recovery were comparable (Rajini & Krishnakumari, 1988a).

Pirimiphos-methyl, 90.5% purity, was fed at dietary concentrations of 0, 1000 or 1500 ppm to groups of 30 male Wistar rats for 28 days. Five rats/group were necropsied 7, 14, 21 or 28 days post-initiation of exposure, and 5 rats/treated group were sacrificed at 35 days (i.e. after 7 days withdrawal from pirimiphos-methyl exposure). The fate of the remaining 5 rats is not reported. Brain and erythrocyte cholinesterase inhibition showed significant dose-related depression at all time intervals during exposure. Erythrocyte cholinesterase was consistent during exposure, but brain cholinesterase did not achieve consistent levels until 14-21 days. Post-exposure recovery occurred in all groups but in brain, cholinesterase activity was still biologically significantly depressed (26 and 28% at 1000 and 1500 ppm, respectively). Plasma cholinesterase activity was variable, but was depressed 17-44% over the various time intervals, the least depression being at the highest dose. Recovery was complete 7 days after cessation of dosing. Non-specific brain carboxylesterase activity was depressed at 1500 ppm at all time intervals, but only after 14 days at 1000 ppm. Recovery was rapid and complete at both dose levels following withdrawal. Plasma non-specific carboxylesterase activity was markedly depressed at all time intervals, but was still significantly depressed following 7 days withdrawal. Renal non-specific carboxylesterase activity was slightly reduced only at 1500 ppm after 14 days treatment and recovered rapidly upon cessation of dosing (Rajini *et al.*, 1989).

Toxicological studies**Acute toxicity studies****Table 1. Acute toxicity of pirimiphos-methyl**

Animal	Sex	Route	LD ₅₀ (mg/kg)	Reference
Mouse	M	oral	1180 (1030-1360)	Clark, 1970
Rat	F	oral	2050 (1840-2260)	Clark, 1970
Rat	M	oral	1861 (1266-2928)	Rajini & Krishnakumari (1988a)
Rat	F	oral	1667 (1187-2284)	Rajini & Krishnakumari (1988a)
Guinea-pig	F	oral	1000-2000	Clark, 1970
Rabbit	M	oral	1150-2300	Clark, 1970
Cat	F	oral	575-1150	Clark, 1970
Dog	M	oral	> 1500	Gage, 1972
Hen	F	oral	30-60	Clark, 1970
Quail	F	oral	~ 140	Gage, 1971a

Test material was 90-94% purity, with at least 14 days post-dosing observation. Toxic signs were typical of those resulting from cholinesterase inhibition.

Table 2. Acute toxicity of metabolites

Compound	Animal	Route	LD ₅₀ (mg/kg)	Reference
2-diethylamino-4-hydroxy-6-methylpyrimidine	Rat	oral	800-1600	Gage, 1971b
2-ethylamino-4-hydroxy-6-methylpyrimidine	Rat (F)	oral	2093 (1841-2380)	Parkinson, 1974
2-amino-4-hydroxy-6-methylpyrimidine	Rat (F)	oral	> 4000	Parkinson, 1974

Toxic signs reported for 2-ethylamino-4-hydroxy-6-methyl pyrimidine comprise urinary incontinence and salivation. No toxic signs were observed with 2-amino-4-hydroxy-6-methylpyrimidine.

Short-term toxicity studies

Rats

Repeated and daily dosing of 10 rats/sex/dose, 5 times weekly for 2 weeks at 200 mg/kg bw/day produced mild signs of poisoning (fibrillation and urinary incontinence) noted only after 7 doses and not increasing in intensity. Body-weight gain was depressed; haemoglobin levels were depressed about 9% and reticulocyte counts were increased - marked anisocytosis and some anisochromia were noted in erythrocytes as well as occasional nucleated erythrocytes and some Howell-Jolly bodies; histopathological changes comprised splenic haematopoiesis and in 1/8 animals, haemosiderosis. A second identical study at 400 mg/kg bw/day resulted in signs of poisoning in 2 days, increasing in severity and resulting in 9/10 deaths in males, and 3/10 deaths in females. Haematology and histopathology were similar to the effects noted at 200 mg/kg bw/day (Clarke, 1970).

Four groups of 25 Alderley Park SPF rats/sex/dose were fed diets containing 0, 8, 80 or 360 ppm 93.1% purity pirimiphos-methyl for 90 days. Twenty/sex/dose were sacrificed at termination of dosing, and the remainder, 28 days later. Body-weight gain in females was reduced 18 and 21%, compared to controls, at 80 and 360 ppm, but food intake in these groups was slightly increased. Plasma cholinesterase was depressed in males (41-72%) and females (56-88%) during weeks 2-12 at 80 and 360 ppm. Recovery to normal activity was observed one week after withdrawal of pirimiphos-methyl. Erythrocyte cholinesterase was depressed in males (39-52%) and females (43-71%) at 360 ppm with incomplete recovery occurring by week one (40% inhibition in males, and 30% inhibition in females). Brain cholinesterase was depressed (mainly in females) at 80 and 360 ppm. Recovery did not occur within the 4-week post-dosing period.

No effects were observed on haematological parameters (haemoglobin concentration, PCV, MCHC, reticulocyte counts, total and differential white cell counts, platelet counts, mean corpuscular diameter and Kaolin-cephalin prothrombin time) or in the incidence of gross or histopathological lesions relative to those observed in controls. The NOAEL was 8 ppm, equivalent to 0.4 mg/kg bw/day (Clapp and Conning, 1970).

Five groups of 12 young male rats (probably Wistar CFT strain, but not specified) were fed dietary concentrations of 0, 10, 250, 500 or 1000 ppm pirimiphos-methyl (90.5% purity) for 28 days. There were no effects on body-weight gain or food intake. Compound intake was calculated to be 0, 4, 100, 200

or 400 mg/kg bw/day (data not given). A slight increase in liver weight was reported at 1000 ppm. No treatment-related pathological changes were observed in liver, brain, lung, heart, adrenal, kidney, spleen or testes. Increased serum transaminases (1000 ppm) and increased alkaline phosphatase (500-1000 ppm) were noted. Hepatic transaminases (β -glucuronidase and alkaline phosphatase) were unaffected. Cholinesterase activity (plasma and brain) were inhibited at 250 ppm and above. The NOAEL appears to be 10 ppm (stated to be equal to 4 mg/kg bw) (Rajini & Krishnakumari, 1988a).

Four groups of male Wistar CFT rats were fed dietary concentrations of 0, 500, 1000 or 1500 ppm pirimiphos-methyl for 28 days. Four rats/group were sacrificed at 7, 14, 21 or 28 days. Mortality, growth rate and food consumption were stated to be unaffected at any dose level. Blood glucose levels in treated rats were consistently below control levels. The authors stated that "decrease in blood glucose level was evident at all dosages during the second week and, at all intervals in 1500 ppm group". This statement is questionable since, when converted to percentage of control values, blood glucose level decrease was greatest in week 1 at 1000 (58%) and 1500 (61%) ppm. Dose-effect relationships are difficult to determine and control values were high (125-138 mg/100 ml). Although standard deviations are given (3.06-9.20), in the absence of individual data, interpretation is equivocal. Blood urea levels were consistently elevated above control levels, but the effect was generally greatest at the mid-dose. Dose/effect relationships do not appear to be present, based on the data available for evaluation. Similarly data on urinary excretion of urea was sporadic and usually non-dose related. Protein excretion in urine was generally increased but, except in week 4, dose/effect relationships are questionable. Creatinine and creatine excretion is equally difficult to interpret (Rajini & Krishnakumari, 1988b).

Four groups of 20 male Wistar CFT rats were administered 0 (coconut oil), 50, 100 or 200 mg 90.5% purity pirimiphos-methyl/kg bw/day, five times weekly for 4 weeks. Four rats/group were sacrificed on days 7, 14, 21 or 28. Signs of toxicity were seen only in week 4 at 200 mg/kg bw/day. Body-weight and food intake were comparable in all groups. Total RBC were depressed at all time intervals and all doses except at 100 ppm and 3 weeks, which were fractionally above control counts. Dose-effect relationships were virtually non-existent despite statistically significant decreases in mean values. A wide variation in control values ($9.95 \times 10^{-6}/\mu\text{l}$ - $8.05 \times 10^{-6}/\mu\text{l}$) also render interpretation difficult, especially with only 4 rats/group.

Similar problems existed in total white cell counts (15 275 - 28 500/ μl in controls over the 4 week period) and differential counts (12 800 - 23 400 for lymphocytes, 1800-4000 for neutrophils). The only consistent effects seem to be the increased clotting time and prothrombin time and decreased platelet counts seen at all dose levels after week 2 (Rajini *et al.*, 1987).

An additional short-term study in rats was reviewed. The purpose of this study was to alleviate concerns that the rats used in the long-term study were rather older (based on their body-weight) than those normally used in such a study. Hence possible effects (especially on cholinesterase activity and inhibition) may have occurred in young rats. To ensure this was not the case, the following study was conducted.

Groups of 12 rats/sex/dose were fed diets containing 0, 5, 8, 10 or 50 ppm 97% purity pirimiphos-methyl for 28 days. The rats were approximately 6 weeks old at receipt and were placed on study over a 3-week period - females during the first week and males during the third week. Plasma and erythrocyte cholinesterase activity were measured on groups of 5 rats/sex/dietary concentration on days -14, -7, 1, 3, 7, 14, 21 and 28 days, and brain cholinesterase was measured on 5 rats/sex/dietary concentration on day 28. There were no effects of pirimiphos-methyl on body-weight gain (animals weighed weekly) or on clinical conditions and behaviour. Food consumption (measured on groups of 3 rats/week) was reduced, males at 5 ppm (statistically significant) and 8 ppm (not statistically significant). At 5 ppm, this reduced food intake was associated with a slight (non-statistically significant) decrease in body-weight gain (ca. 5%). Food utilization was comparable in all groups. At termination, gross pathology of rat(s)/sex/group did not reveal any lesions attributable to pirimiphos-methyl. Plasma cholinesterase depression consistently exceeded 20% in the 50 ppm group. Sporadic inhibition was noted at 8 and 10 ppm, as was sporadic elevation. Erythrocyte cholinesterase inhibition was unaffected by pirimiphos-methyl even at 50 ppm. Brain cholinesterase inhibition exceeded 10% in both sexes at 50 ppm but was not significantly affected at lower dietary concentrations (Berry & Gore, 1975).

Dogs

Four groups of beagle dogs/sex were fed gelatin capsules at pirimiphos-methyl doses of 0, 2, 10, or 25 mg/kg bw/day, for 13 weeks. Two dogs/sex/dose were sacrificed at 13 weeks, and the remainder at 17 weeks. Clinical signs (dry skin, dull coat during weeks 2 and 3, and increased incidence of vomiting during the first 5-6 weeks) were observed at 25 mg/kg bw/day. An increased frequency of liquid stools was noted at 10 and 25 mg/kg bw/day. Body-weight gain was reduced in both sexes at 25 mg/kg bw/day and in females at 10 mg/kg bw/day. At 2 mg/kg bw/day, female body-weight gain reduction was of borderline significance. Reduced food intake (dose-related) occurred in all female test groups and in males at 25 mg/kg bw/day. At 2 and 10 mg/kg bw/day male food intake was inconsistent. Heart rate was reduced (both sexes) at 25 mg/kg bw/day. Plasma cholinesterase was depressed at all dose levels (more than 20%) but recovery was rapid following cessation of dosing. Erythrocyte cholinesterase activity was depressed in a dose-related pattern, the onset being late (10-12 weeks) at 2 mg/kg bw/day. At 10 and 25 mg/kg bw/day, inhibition increased with time. Terminal brain cholinesterase activity at 13 and 17 weeks was comparable to controls in all groups. Two dogs showed high ALAT and SAP levels after 3

months dosing, and a third, a less marked ALAT increase at 25 mg/kg bw/day. Bile duct proliferation and portal cirrhosis occurred in 1/2 males at 25 mg/kg bw/day, and bile duct proliferation only in 1/2 males at 10 mg/kg bw/day at 13 weeks. After withdrawal, minimal bile duct proliferation occurred in 1/2 dogs of each sex at 25 mg/kg bw/day. The NOAEL was 2 mg/kg bw/day, based on reduced body weight gain in females at 10 mg/kg bw/day (Noel *et al.*, 1970).

Four groups of 4 beagle dogs/sex were dosed with 0, 0.5, 2 or 10 mg/kg bw/day via gelatin capsules for 2 years (corn oil solution). A 7-day break in dosing occurred at 140 days (due to problems with liquefying the test material). Brain cholinesterase activity was 81, 78 and 44% of control values at 0.5, 2 and 10 mg/kg bw/day, respectively. Erythrocyte cholinesterase activity was depressed with respect to pre-dosing levels at 2 mg/kg bw/day (greater than 20% from week 12 onwards) and 10 mg/kg bw/day. Plasma cholinesterase activity depression was 21-33% almost consistently throughout the study at 10 mg/kg bw/day, 21-33% from week 4 and frequently thereafter at 2 mg/kg bw/day, and infrequently (weeks 38, 77, and 102) by 20-25% at 0.5 mg/kg bw/day. One female dog died on day 401 after showing few clinical signs of intoxication. At 10 mg/kg bw/day, reduced food intake and body-weight gain were noted. Electrocardiogram records and ophthalmoscopy were normal in all groups as were haematological parameters (erythrocyte counts, Hb, PCV, HCHE, MCV, reticulocyte counts, total and differential white cell counts, platelet counts and prothrombin index), and clinical chemistry (urea, glucose, protein, SAP, K⁺ and Na⁺) except for a slight increase in ALAT at 12, 26 and 38 weeks in some dogs at 10 mg/kg bw/day. Histopathological changes were comparable in all groups although absolute and relative (to body-weight) liver weights were increased at 10 mg/kg bw/day (Rivett *et al.*, 1973). The 1974 JMPR (Annex 1, reference 23) did not appear to consider the 19% depression of brain cholinesterase activity at 0.5 mg/kg bw/day to be toxicologically significant.

Subsequent data have been submitted (ICI, 1988) which comprise historical control data on dog studies performed between 1970 and 1975. These data clearly indicate that the apparent brain cholinesterase inhibition at 0.5 and 2 mg/kg bw/day are well within historical control values, based on 8 studies from the same laboratory, using the same measurement techniques. Concurrent controls in the pirimiphos-methyl study were, in 2 males and 3 females, abnormally high. The Meeting concluded that the NOAEL was 2 mg/kg bw/day, based on reduced cholinesterase activity and reduced body weight gain at 10 mg/kg bw/day.

The potential hepatic effect of pirimiphos-methyl on dog liver was investigated using 8 male dogs dosed by capsule at 25 mg/kg bw/day, and 2 male dogs dosed at 25 mg/kg bw/day for 8 weeks and then increasing to 35 mg/kg bw/day (14 days), 45 mg/kg bw/day (7 days), and finally 50 mg/kg bw/day (17 days). Six control animals received corn oil only. Four out of eight dogs at 25 mg/kg were withdrawn from treatment for 2 days after week 3 because of toxic signs. During week 4, 3/8 dogs were sacrificed (due to weight loss) and during

week 12, 1/2 dogs, after 7 days at 50 mg/kg bw/day was taken off treatment for 5 days. Slight bile-duct proliferation was seen in 2 dogs receiving 25 mg/kg bw/day for 13 weeks but not in any other animals, even at 50 mg/kg bw/day. Investigation of plasma alkaline phosphatase, plasma alanine aminotransferase, aspartate aminotransferase, and plasma glutamate dehydrogenase showed elevation of these enzymes in almost all test animals on occasions, throughout the study. No correlation with histopathology was apparent. Individual sensitivity was extremely variable (Garuti *et al.*, 1976).

Long-term toxicity/carcinogenicity studies

Mice

Three groups of 52 CFLP mice/sex were fed dietary concentrations of 0, 5 or 250 ppm 97.8% purity pirimiphos-methyl for 80 weeks. A fourth group was fed 300 ppm increasing weekly by 50 ppm to a final concentration of 500 ppm. Satellite groups of 12 mice/sex fed 0, 5 and 500 ppm were utilized for blood cholinesterase studies. Mortality was not significantly affected. Body-weight gain was not affected overall, although food consumption was slightly reduced in females at 500 ppm over the 80 week period. Water consumption was unaffected. Erythrocyte cholinesterase activity (measured in weeks 0, 12, 36 and 80) was depressed in males at 5 ppm in week 36 only, and in both sexes at all time intervals at 500 ppm. Plasma cholinesterase activity was significantly decreased (> 20%) at all time intervals at 500 ppm, and at 5 ppm in males at weeks 12 and 36, and in females at week 80. Histopathological changes (hepatocytic vacuolation hepatic nodular hypoplasia in females only, renal foci of lymphocytic infiltration, perivascular or peribronchial lymphoid aggregations in the lung and abscesses and small haemorrhages in the ovaries were not deemed to be of toxicological significance since they occurred in all groups and no dose-relationship was apparent. There was no evidence of tumour induction at dose levels up to 500 ppm pirimiphos-methyl. The NOAEL was 5 ppm, equal to 0.5 mg/kg bw/day in male mice, and 0.6 mg/kg bw/day in female mice (Hunter *et al.*, 1976).

Rats

Four groups of 49 Wistar SPF rats/sex were fed pirimiphos-methyl at dietary concentrations of 0, 10, 50 or 300 ppm for 2 years. An additional 24 rats/sex/group were fed the same diets and were sacrificed (8/sex/group) at 12, 26 and 52 weeks for brain cholinesterase studies. At termination of dosing, 8 rats/sex/group were fed control diet for 4-8 weeks prior to sacrifice, all other rats were sacrificed at 2 years. Survival (33-56%) was unaffected by pirimiphos-methyl at dose levels up to 300 ppm. Two outbreaks of infection were reported - one at 16 weeks, when "several animals of either sex developed swellings (lasting no more than 72 h) in the area of the salivary glands". No changes in behaviour, appetite or condition were observed. The condition, distributed between groups, did not recur. The second outbreak, occurring in the latter weeks of the test, was a

respiratory disease, resulting in several deaths. All surviving rats were treated with 18 mg oxytetracycline/kg bw/day for 5 consecutive days during week 86.

Body-weight gain and food intake were comparable in all groups. At 300 ppm, marked plasma cholinesterase inhibition (50-80%) and some inhibition of erythrocyte and brain cholinesterase (20-40%) were noted. The only other adverse effect at this dietary concentration was slight anaemia in female rats. Only female rat plasma cholinesterase was consistently inhibited (50-65%) at the 50 ppm dietary level. Brain cholinesterase was reduced (> 20%) in males at weeks 26 and 104 at 50 ppm. Slight concurrent plasma cholinesterase inhibition occurred in females at 10 ppm. Brain erythrocyte cholinesterase was not affected at this dietary concentration. Recovery of depressed cholinesterase activity was usually complete following the 8-week withdrawal period. Organ weights and weight ratios, and incidence of gross or histopathological changes were comparable between groups. Tumour incidences in treated groups were generally comparable to incidences in control groups. The NOAEL was 10 ppm, equivalent to 0.5 mg/kg bw/day (Gore *et al.*, 1974a).

Reproduction studies

Rats

A multigeneration study (3 generations, 2 litters/generation using b litters for parental animals) using groups of 24 female and 12 male Charles River CD rats fed diets containing 0, 20 or 200 ppm nominal concentrations was performed. Since dietary analysis indicated levels of only 3-8.7 ppm were, in fact, present in the diet of the F₀ generation (nominal level 20 ppm), the study at this dose level was extended to produce a 4th generation (a and b litters).

No adverse effects on parental mortality, body-weight, or gross pathology were seen in any test group. Neither were toxic signs observed. At 200 ppm, mating performance and pregnancy rate were reduced in the production of F₂-F₃ litters and in F₃ litters at 20 ppm. The decreased pregnancy rate was dose-related. Litter data (total litter losses, litter size, litter and mean pup weights, pup mortality, and length of gestation) were comparable between groups. Examination of the ultimate litters (F_{3b} at 200 ppm and F_{4b} at 20 ppm) by organ weight analyses, skeletal staining of 10/pups/sex from each group, and histopathology of 10 pups/sex from 0 and 200 ppm dose levels did not reveal any compound-related effects. A NOAEL was not demonstrated in this study (Palmer & James, 1972).

The testes of the F_{1b} and F_{2b} male rats were examined histopathologically in an attempt to find an explanation for their reduced mating performance. However, in the testes examined from rats of the control and both treatment groups, the degree of activity and maturity of the process of spermatogenesis were comparable (Annex 1, reference 23).

Groups of 20 SPF CF strain rats/dose level were fed dietary concentrations of 0, 5, 10 or 100 ppm continuously for 3 generations (1 litter/generation). There were no consistent effects on parental animals as assessed by toxic signs, mortality, food consumption, body-weight, mating performance, pregnancy rate or duration of gestation. Parental plasma and erythrocyte cholinesterase depression ($\geq 20\%$) was recorded in one or both sexes at 100 ppm after 7-9 weeks on diet (i.e. during the pre-mating period). No effects were observed in pups as determined by litter size, pup mortality, litter or mean pup weights, gross pathology of F_{3a} or incidence of anomalies. The NOAEL was 100 ppm, equivalent to 5 mg/kg bw/day (Palmer & Hill, 1976).

Special studies on potentiation

Combined administration of half LD₅₀ dose of pirimiphos-methyl with either gamma-BHC or dichlorvos to rats did not indicate potentiation (Annex 1, reference 23). However, similar studies with bioresmethrin indicated some possible potentiation but to an extent which does not appear to be sufficient to be of practical significance (Annex 1, reference 27).

Special study on mammalian cell transformation

An *in vitro* study utilizing Syrian hamster kidney cells (fibroblast morphology, cell line BHK21/c13) exposed to doses ranging from 0.23 to 2300 µg/ml, according to the method of Styles, 1977) failed to induce cell transformation (Trueman, 1983).

Special studies on teratogenicity

Rats

Three groups of 18-21 pregnant Alderby Park SPF rats were fed diets of 0, 10 or 200 ppm from gestation days 1 through 20 (day of insemination: day 0). Pups were removed by caesarean section on day 20. Maternal body-weight gain was slightly reduced on day 7 of gestation, but was normal by day 20. No differences in food consumption or gross pathology of the dams were observed. Implants/dam, resorption incidence, sex ratio, and incidence of malformations were within the normal range of distribution. At 200 ppm, mean fetal weight was decreased, but this was probably a consequence of a concomitant increase in litter size (Hodge and Moore, 1972).

A second rat study used 24 Alpk:AP Wistar derived rats/dose level. Rats were administered 0, 1.5, 15 or 150 mg pirimiphos-methyl (purity 88.5% w/w, doses corrected for 90.9% purity) in corn oil/kg bw/day by gavage on gestation days 7-16 inclusive (day 1 being the day of confirmation of mating) indicated maternal toxicity (changes in chemical signs and one death), decreased body-weight gain and food intake were observed at 150 mg/kg bw/day. In the 1.5 mg/kg bw/day pre-

implantation losses were increased with concomitant effects on numbers of live pups and mean gravid uterine and litter weights. Values were, however, stated to be within normal ranges for the strain. It is improbable that this observation is compound-related because of the absence of similar findings at higher doses - and the minimal exposure which may have occurred prior to implantation. No compound- or dose-related effects on pup weight, *in utero* survival or incidence of malformations/variants were noted except for slight evidence of delayed ossification in *pes* scores at 150 mg/kg bw/day. NOAELs for maternal toxicity (15 mg/kg bw/day), embryotoxicity (15 mg/kg bw/day, based on reduced *pes* scores) and teratogenicity (≤ 150 mg/kg bw/day) were identified (ICI, 1985).

Rabbits

Three groups of 16 or 17 artificially inseminated Dutch rabbits were orally administered gelatin capsules containing corn-oil solutions of pirimiphos-methyl to give 0, 1 or 16 mg/kg bw/day from gestation days 1 to 28 inclusive. Three does died during the study; two in the 1 mg/kg bw/day group, and one in the 16 mg/kg bw/day group. Litter data, however, were only available in summary format, on 10, 10 and 11 does at 0, 1 and 16 mg/kg bw/day respectively, despite 16, 15 and 15 does surviving to term. Whether this discrepancy is due to unsuccessful fertilization or pre-implantation loss or total resorption of litters cannot be ascertained. Based on the available data, mean number of implants/reported litter were similar in all groups, and resorption rates were highest in the control group. Litter size was increased at 16 mg/kg bw/day, and mean litter weight was slightly depressed at this dose level. Sex ratio (M/F) appears to increase with dose, but are stated (no data) to be within normal ranges. One high-dose fetus, with placental thrombosis and necrosis, showed multiple malformations. A dose-related increase in the occurrence of 14 caudal vertebrae was noted. Cholinesterase depression occurred in erythrocytes at 1 (23%) and 16 (32-60%) mg/kg bw/day and in plasma at 16 mg/kg bw/day (23%). The NOAEL for embryofetal toxicity and teratogenicity was 16 mg/kg bw/day. A NOAEL for maternal toxicity was not demonstrated since at 1 mg/kg bw/day, erythrocyte cholinesterase inhibition exceeded 20% (Gore *et al.*, 1974b).

Special studies on neurotoxicity

Hens

In a study designed to assess acute delayed neurotoxicity, pirimiphos-methyl was administered by oral intubation as a solution in arachid oil to groups of five Light-sussex adult hens at dose levels of 20, 30, 40, 50 or 60 mg/kg bw. The LD₅₀ was established at approximately 79 mg/kg bw. Over an observation period of 21 days post-dosing none of the animals showed signs of neurotoxicity. At day 26 the surviving birds in the two highest dose groups (4/5 at 50 mg/kg bw and 2/5 at 60 mg/kg bw) were re-dosed after being protected with intramuscular injection of atropine (10 mg/kg bw) and 2-PAM (50 mg/kg bw) and observed for a further 21

days. Group sizes were made up to 5 with "new" protected birds which received a single dose of pirimiphos-methyl. A further group of 5 birds were dosed with 500 mg TOCP/kg bw as a positive control. Clinical observations were confined to two birds in the 50 mg/kg bw dose group which showed sporadic doubtful signs of incoordination. Surviving birds (2/5) in 60 mg/kg bw dose group showed no signs of neuropathological changes. All birds in the positive control group showed signs of ataxia and significant neuropathological changes. It is concluded that pirimiphos-methyl does not cause delayed neurotoxicity following acute doses up to 60 mg/kg bw (Annex I, ref. 27; Ross *et al.*, 1975).

In a study designed to examine the potential of pirimiphos-methyl to cause delayed neurotoxicity after subchronic exposure, groups of ten adult hens were dosed at 0 (untreated control), 0 (corn oil control), 0.5, 1.0, 2.5, 5.0 or 10 mg pirimiphos-methyl (93.5% purity) kg bw/day by oral intubation for 90 days. A further group of ten birds received 90 daily doses of 7.5 mg TOCP/kg bw as a positive control. A further 2 groups of 10 hens received 90 doses of either 5 or 10 mg pirimiphos-methyl/kg bw/day followed by a recovery period of 90 days when the birds were not dosed. Dosing was discontinued for birds in the two recovery groups when signs of toxicity were severe and restarted when signs of recovery were noted. The final recovery period was started when a bird had received a total of 90 doses.

Analysis of test solutions indicated concentrations were within 8% of nominal values, and were stable up to 10 days. Mortalities were 1/10, 3/10 and 4/10 in the main groups at 1.0, 2.5, 5.0 and 10 mg/kg bw/day; 1/10 and 4/10 in the 5 and 10 mg/kg bw/day ancillary groups during treatment. Post-treatment deaths, during the recovery period were 4/9 and 1/6 at 5 and 10 mg/kg bw/day, respectively. Signs of toxicity, incidence and severity increasing with dose, were observed at 1.0 mg/kg bw/day and above. There were no signs of ataxia occurring as a result of delayed neurotoxicity except in the positive control group (7/10 birds). Food intake was variable during dosing, but overall indicated a dose-related decrease at 10 mg/kg bw/day, and in the positive control group. Food intake increased in the recovery groups after termination of dosing. Mean body-weight values were decreased in one group at 5 mg/kg bw/day and both groups at 10 mg/kg bw/day of dosing. Degenerative damages in nerve tissues at histopathological examination were only observed in the positive control group. The study did not indicate any evidence of delayed neurotoxicity due to exposure of adult hens to toxic doses of pirimiphos-methyl (Roberts *et al.*, 1983).

A pilot study using 3 groups of 6 ISA hens protected with 50 mg/kg bw 2-PAM i.p. and 10 mg atropine sulphate/kg bw i.p. at dose levels of 0, 100 or 117 mg pirimiphos-methyl/kg bw/p.o. with 14 day post-dosing observation determined a dose of 100 mg pirimiphos-methyl/kg bw to be a suitable dose for protected hens in a delayed neuropathy assessment study.

Enzyme activity (NTE and AChE) were determined in hens given corn oil (4 birds), TOCP (2 birds), protected hens given 100 mg pirimiphos-methyl/kg bw (10 birds), p.o. with sacrifice at 24 h (2 control, 1 TOCP and 3 treated birds) and 48 h (similar numbers). NTE was unaffected in control and pirimiphos-methyl dosed birds, but markedly inhibited in those given TOCP at both 24 and 48 h. Conversely, TOCP had minimal effect on brain or spinal cord acetylcholinesterase, which was markedly inhibited by pirimiphos-methyl.

In the main study, 10 hens were given corn oil, 10 TOCP, and 40 received 100 mg pirimiphos-methyl in corn oil/kg bw p.o., the dose being repeated after 21 days in control and pirimiphos-methyl treated birds. TOCP birds were sacrificed at 21 days, and the others at 42 days. Delayed ataxia was only seen in TOCP treated birds. Control and pirimiphos-methyl treated birds showed similar spinal cord and peripheral nerve histopathology, whereas TOCP showed disruption, fragmentation and distortion of some spinal cord axon with some damage to the myelin sheath or peripheral nerves as well as axonal degeneration (Lock & Johnson, 1990; Hawkin *et al.*, 1989).

Special studies on genotoxicity

The results of genotoxicity studies on pirimiphos-methyl are summarized in Table 3.

Table 3. Results of genotoxicity assays on pirimiphos-methyl

Test	Dose	Result	Reference
Male mouse dominated lethal	15, 80 or 150 mg/kg bw/day for 5 days prior to pairing	Pregnancy frequency reduced at 150 mg/kg bw	McGregor, 1975
<i>Salmonella typhimurium</i> (No metabolic activation)	ditto	+	Hanna & Dyer, 1975
<i>Escherichia coli</i> (No metabolic activation)	ditto	-	Hanna & Dyer, 1975
Micronucleus	ditto	-	Seiber (1975)
Micronucleus (mouse)	100, 200 and 400 mg/kg bw	-	Rajini <i>et al.</i> (1986)
Sperm-morphology (mouse)	100, 200 and 400 mg/kg bw	-	Rajini <i>et al.</i> (1986)
Bone marrow cytogenetic study (rat)	Single dose 32, 102, 320 mg/kg bw	-	Done & McGregor (1980)
Bone marrow cytogenetic study (rat)	5 consecutive daily 32, 102, 320 mg/kg bw	- ve at 32 and 102 mg/kg bw. At 320 mg/kg bw/day, chromatid gap incidence was increased and one chromosome gap was recorded	Done & McGregor (1980)
<i>Salmonella typhimurium</i> TA1535, TA1537, TA1538, TA98 and TA100 (with and without metabolic activation)	1.6-5000 µg/plate	-	Callander (1984)
Mouse lymphoma cell assay (LS178Y cells) (with and without metabolic activation)	12.5-200 µg/ml	-	Cross (1986)
Human lymphocyte cytogenetics (with and without metabolic activation)			
DONOR 1	12, 58, 116 µg/ml**	-	Wildgoose <i>et al.</i> (1986)
DONOR 2	12, 29, 58 µg/ml**	-	
Hamster sister chromatid exchange in lung fibroblasts			
with metabolic activation	0.14, 0.29, 1.4, 2.9, 14, 29	+ - ***	Howard <i>et al.</i> (1986)
without metabolic activation	0.14, 0.29, 1.4, 2.9, 14, 29,	+ -****	
activation	145 µg/ml		

* The doses in this study tended to be low and metaphase chromosome spread was variable, resulting in reduced numbers of cells available for scoring.

** Maximum tolerated doses for the different donors.

*** Positive results (dose-related) were noted at 14, 29 and 145 µg/ml. At 145 µg/ml, the assessment was based on a single cell culture at a dose which exhibited marked toxicity.

**** Positive results were noted at all dose levels except 2.9 µg/ml. Dose/effect relationships were not clearly established.

Observations in humans

A dose of 0.25 mg 97.8% purity pirimiphos-methyl/kg bw/day was taken orally for 28 days by 5 healthy males (59.5-73 kg bw, 25-45 years old). Blood samples were taken on days -14, -7, 1, 3, 7, 14, 21 and 28. One subject showed plasma cholinesterase inhibition (21.5%) on day 28. Otherwise variations, both above and below pre-dosing values, were within 12%. Four of five subjects had red-cell cholinesterase activity values slightly below the pre-exposure values during the last 2 weeks of the study. However, the group means for each time interval did not differ significantly and the variations noted were within the range of variations found by others for normal untreated subjects (Chart *et al*, 1974).

Three male (62-73 kg, 22-27 years old) and 4 females (44-60 kg, 21-49 years old) were given 0.25 mg 97.8% purity pirimiphos-methyl/kg bw/day by capsule for 56 days. Blood samples were taken twice prior to initiation of dosing, and on days 7, 14, 21, 28, 35, 42, 49 and 56 of the study and also in the recovery period 7, 14, 21 and 29 post-treatment. Controls comprised 2 females (44 and 46 kg, aged 29 and 30). No compound-related effects were observed on liver function (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and glutanyl transpeptidase in plasma), haematology (Hb, PCV, MCHC, total and differential WBC, platelets, ESR) or erythrocyte cholinesterase. Plasma cholinesterase was depressed about 20% in 2/4 females on days 14, 21 and 28 and in one female on days 28 and 35. The effect did not increase with time. All values were normal during the withdrawal period (Howard & Gore, 1976).

Two groups of spray workers (5/group) together with 1 mixer-loader per group applied actellic 50 EC to grain using a compression sprayer, hand held, for 3 consecutive days (4-5 h/day). The formulation, containing 50% pirimiphos-methyl, was sprayed at a concentration of 5 g/l with an application rate of 0.158 a.i./m². A second study, using similar groups applied actellic 40 EC containing 40% pirimiphos-methyl sprayed at a concentration of 46 g/l and application rate of 2.0 a.i./m² in unoccupied dwellings for mosquito control. In the two studies, spray workers in one group were in normal clothing, in the second group, protective clothing (cotton overalls, caps, canvas shoes and safety glasses, and in the case of the dwelling spray workers, cotton masks). Mixer-loaders wore rubber boots, gloves, caps, cotton jackets, cotton masks and safety glasses. Cholinesterase (blood and plasma) was measured on 3 consecutive days prior to spraying, at lunchtime and the evening on the days of spraying, and on days 1, 2, 3 and 10 post-spraying. Clinical examinations were performed by registered practitioners and comprised case histories and a general medical examination with emphasis on gastrointestinal, neuromuscular, cardiorespiratory, visual and psychological effects and peripheral and central nervous systems prior to, during, and subsequent to spraying. Cholinesterase depression occurred on a group basis in all groups over the spray period, with full recovery within 24 h post-dosing. The greatest depression occurred in workers applying the 40 EC formulation. Ten of the 24 exposed individuals showed > 15% depression of erythrocyte cholinesterase, the

maximum depression being 23%. Levels of plasma cholinesterase inhibition were inconsistent, and less than those seen for erythrocyte cholinesterase inhibition. There were no clinical manifestations of toxicity (Chester & Hart, 1986).

COMMENTS

Following oral administration of pirimiphos-methyl to male rats, 80.7% and 7.3% of the administered dose were excreted via urine and faeces, respectively, within 24 h. In the dog, 48 h after dosing with either 18.4 or 16.7 mg/kg bw, urinary excretion was 64.4% or 82.5% and faecal excretion 17.3% or 13.3%.

Metabolic data indicated that the P-O-C bond of pirimiphos-methyl was readily cleaved and that N-de-ethylation and/or conjugation were further steps in the metabolism of the pyrimidine leaving group. Although the oxygen analogue of pirimiphos-methyl was not detected as a urinary metabolite, the fact that cholinesterase inhibition occurred *in vivo* suggests that the oxygen analogue was also formed and may be an intermediate step leading to the identified urinary products.

In rats and dogs 2-ethylamino-4-hydroxy-6-methylpyrimidine was the major metabolite (30% of the administered dose).

The oral toxicity of pirimiphos-methyl is low. WHO has classified the compound as slightly hazardous (WHO, 1992).

The only biochemical effect consistently observed with pirimiphos-methyl in acute short-term or long-term studies was cholinesterase inhibition.

In a series of short-term rat studies at dose levels of 0, 8, 80 or 360 ppm for three months, 0, 10, 250, 500 or 1000 ppm for 28 days, 200 mg/kg bw five times weekly for 14 days, and 0, 5, 8, 10 or 50 ppm for 28 days (young rats) the overall NOAEL was 10 ppm (equivalent to 0.5 mg/kg bw/day) with effects on erythrocyte cholinesterase and brain acetylcholinesterase at 80 ppm. At high dose levels (200 mg/kg bw, five times weekly for two weeks) erythrocyte morphology was affected. The NOAEL in young rats was also 10 ppm, with brain (but not erythrocyte) acetylcholinesterase depressed at 50 ppm after 28 days.

In two dog studies (13 weeks at dose levels of 0, 2, 10 or 25 mg/kg bw/day via capsule and 0, 0.5, 2 or 10 mg/kg bw/day for two years by capsules) the NOAEL was 2 mg/kg bw/day, based on brain acetylcholinesterase inhibition.

In an 80-week study in mice at dietary concentrations of 0, 5, 250 or 500 ppm, a NOAEL based on blood cholinesterase depression was 5 ppm (equal to 0.5 mg/kg bw/day) (blood cholinesterase was not measured at 250 ppm, only at 5 and 500 ppm). Pirimiphos-methyl was not carcinogenic in mice.

In a two-year study in rats at dietary concentrations of 0, 10, 50 or 300 ppm, tumour incidence was comparable to controls. The NOAEL was 10 ppm (equivalent to 0.5 mg/kg bw/day) with brain acetylcholinesterase inhibition occurring at higher levels. Pirimiphos-methyl was not carcinogenic in rats.

In a four-generation reproduction study in rats at nominal dietary concentrations of 0, 20 or 200 ppm, dose-related reduction of pregnancy rates and reduced mating performance at 200 ppm were noted. Dietary analyses indicated that the 20 ppm diet only contained 9 ppm pirimiphos-methyl. No NOAEL was demonstrated in this study.

A repeat study at dietary concentrations of 0, 5, 10 or 100 ppm for three-generations (1 litter/generation) did not show any adverse effects on reproductive parameters at any dose level. The NOAEL was 100 ppm (equivalent to 5 mg/kg bw/day) for reproductive effects.

In two rat teratology studies, one at dietary concentrations of 0, 10, or 200 ppm and the second at dose levels of 0, 1.5, 15, or 150 mg/kg bw/day, dosing extending over or beyond the period of embryogenesis did not demonstrate any evidence of teratogenicity. Fetotoxicity was observed at 200 ppm (equivalent to 10 mg/kg bw/day) and 150 mg/kg bw/day. NOAELs for maternal toxicity (15 mg/kg bw/day), embryotoxicity (15 mg/kg bw/day) and teratogenicity (\leq 150 mg/kg bw/day) were identified.

A rabbit teratogenicity study at doses of 0, 1 or 16 mg/kg bw/day administered from days 1-28 of gestation did not show any evidence of teratogenic effects. The NOAEL for fetotoxicity and teratogenicity was 16 mg/kg bw/day.

Four studies in hens indicated that pirimiphos-methyl does not cause delayed neurotoxicity.

After considering the available *in vitro* and *in vivo* genotoxicity data, the Meeting concluded that pirimiphos-methyl was not genotoxic.

In two experimental studies with human volunteers of 28 and 56 days, the highest dose tested in both studies (0.25 mg/kg bw/day) failed to induced erythrocyte cholinesterase inhibition in either study.

In determining the ADI the first multigeneration study in rats was discarded because the dietary concentrations were uncertain, and the adverse effects noted (decreased pregnancy rate and mating performance) were atypical of those normally seen in reproduction studies with organophosphorous esters (decreased pup weight gain and pup mortality during early lactation). A clear NOAEL of 100 ppm (equivalent to 5 mg/kg bw/day, the highest dose tested) was demonstrated in the repeat study.

Studies with mice, rats, and dogs, showed NOAELs of 0.5 mg/kg bw/day or above. In human studies, no cholinesterase inhibition was seen at 0.25 mg/kg bw/day (the highest dose tested). On this basis, the Meeting revised the ADI to 0.03 mg/kg bw/day by applying a 10-fold safety factor to the NOAEL in the human studies.

TOXICOLOGICAL EVALUATION

Level causing no toxicological effect

Mouse:	5 ppm, equal to 0.5 mg/kg bw/day (80-week study)
Rat:	10 ppm, equivalent to 0.5 mg/kg bw/day (two-year study)
	100 ppm, equivalent to 5 mg/kg bw/day (three-generation reproduction study)
Dog:	2 mg/kg bw/day (two-year study)
Humans:	0.25 mg/kg bw/day

Estimate of acceptable daily intake for humans

0-0.03 mg/kg bw

Studies which will provide information valuable in the continued evaluation of the compound

Further observations in humans.

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