PIRIMIPHOS-METHYL (ADDENDUM)

First draft prepared by
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Explanation

Pirimiphos-methyl is an organophosphorus insecticide and acaricide. Toxicological monographs for pirimiphos-methyl were prepared by the Joint Meeting in 1974, 1976 and 1992. In 1992, an acceptable daily intake (ADI) of 0–0.03 mg/kg bw was established based on a NOAEL of 0.25 mg/kg bw per day in a 28-day and a 58-day study in human volunteers, and a safety factor of 10.

At the request of the Codex Committee on Pesticide Residues (CCPR), the requirement for an acute reference dose (ARfD) was considered on the basis of data from previous evaluations as well as new studies. A number of studies previously evaluated by the JMPR were considered to be possibly relevant for establishing an ARfD and were re-evaluated.

Pirimiphos-methyl was being considered by WHO as a larvicide treatment for drinking-water. For that reason, the WHO programme on guidelines for drinking-water quality had recommended that pirimiphos-methyl be evaluated toxicologically by JMPR.

For pirimiphos-methyl, the specifications were established by the Joint FAO/WHO Meeting on Pesticide Specifications (JMPS) and published as *WHO specifications and evaluations for public health pesticides: pirimiphos-methyl* (2006).[1]

The pivotal studies with pirimiphos-methyl were certified as being compliant with good laboratory practice (GLP). Other available studies were carried out before the Organisation for

[1]Available from: http://www.who.int/whopes/quality/en/Pirimiphos_methyl_eval_may_06.pdf.

Economic Co-operation and Development (OECD) guidelines on GLP were implemented. However, the quality of these studies was considered to be acceptable.

Evaluation for an acute reference dose

1. Biochemical aspects

1.1 Absorption, distribution and excretion

No studies of absorption distribution and excretion were available for the present evaluation. A number of relevant summaries from the 1992 JMPR evaluation are presented below; an asterisk is used to indicate that the text describing these studies was extracted from the JMPR 1992 report.

Rats

Oral administration of 2-^{14}C-ring-labelled pirimiphos-methyl at a dose of 0.6 mg/kg bw to five 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 (unidentified) metabolites were present in the urine (Bratt & Dudley, 1970).

Female rats given 2-^{14}C-pirimiphos-methyl at a dose of 7.5 mg/kg bw orally were bled (cardiac puncture, three rats per time interval) at 0.5, 1, 3, 5, 7 or 24 h after dosing. Maximum blood concentrations (at 0.5 h) were 2–3 µg/ml, declining by 50% 1 h after dosing. By 24 h, concentrations of ^{14}C in blood were 0.2–0.3 µg/ml, and of pirimiphos-methyl, 0.01–0.02 µg/ml. Rats treated for 4 days with 2-^{14}C-pirimiphos-methyl at a dose of 7.5 mg/kg bw per day and sacrificed at intervals of 24 h did not show any increase in blood concentrations with time. Tissue concentrations of total radioactivity in the liver, kidney and fat over the 4 days were generally less than 2 mg pirimiphos-methyl equivalents/kg tissue (concentrations 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 ^{14}C-labelled pirimiphos-methyl at a dose of 1 mg/kg bw per day. Four groups of three animals were dosed for 3, 7, 14 or 21 days and sacrificed 24 h after the final dose. A further five groups of three rats were given similar doses for 28 days and sacrificed 1, 3, 7, 14, or 28 days after dosing. For each of the nine groups, one rat that did not receive pirimiphos-methyl was used as a control. After sacrifice, samples of liver, kidney, muscle, fat, erythrocytes and plasma were taken for analyses. Urine and faeces were collected from two rats during the 24 h after the seventh dose. Recovery of ^{14}C from ^{14}C-labelled pirimiphos-methyl added to control tissues was 96.9 ± 5.2%. In all tissue samples taken at all time intervals, the concentration of radioactivity was very low, close to or below detection limits. Concentrations did not increase with repeated dosing. Liver concentrations were fairly constant (0.03 ppm) and similar concentrations were detected in some kidney samples. In other tissues, the concentration of radioactivity was generally below the limits of detection (0.04–0.06 ppm). Three days after cessation of dosing, one animal had detectable concentrations of radioactivity in the kidney. At 7 days and on subsequent days, no residues were found. Excretion was between 70% and 80% of a single dose, after administration of seven consecutive doses, providing evidence for rapid metabolism and elimination rather than poor absorption (Hawkins & Moore, 1979).

Dogs

Groups of one male beagle dog were given capsules containing 2-^{14}C-ring-labelled pirimiphos-methyl at a dose of 18.4 or 16.7 mg/kg bw. Of the administered dose, 64.4% or 82.5% was excreted in the urine, and 17.3% or 13.3% in the faeces, respectively, in 48 h. Nine (unidentified) metabolites were present in the urine (Bratt & Dudley, 1970).

As a thiophosphate, pirimiphos-methyl requires metabolic activation (from P=S to P=O) to inhibit acetylcholinesterase. No data are available on the interindividual variability of P=S oxidation (Tang et al., 2005). Pirimiphos-methyl is highly lipophilic (log K_{ow} = 4.2).

2. Toxicological studies

2.1 Acute toxicity

The acute oral toxicity of pirimiphos-methyl is summarized in Table 1.

Table 1. Results of studies of acute toxicity with pirimiphos-methyl

Species	Strain	Sex	Route	Purity (%)	LD_{50} (mg/kg bw)	Reference
Mouse	Unknown	Male	Oral	90–94	1180	Clark (1970)
Rat	Unknown	Female	Oral	90–94	2050	Clark (1970)
Rat	Unknown	Male	Oral	90.5	1861	Rajini & Krishnakumari (1988)
		Female	Oral		1667	
Guinea-pig	Unknown	Female	Oral	90–94	1000–2000	Clark (1970)
Rabbit	Unknown	Male	Oral	90–94	1150–2300	Clark (1970)
Cat	Unknown	Female	Oral	90–94	575–1150	Clark (1970)
Dog[a]	Unknown	Male	Oral	90–94	> 1500	Gage (1972)

[a] The study of acute toxicity in dogs, by Gage (1972), was not available for the present evaluation. Data were taken from JMPR 1992.

The clinical signs reported in the studies of acute toxicity, as described by Clark (1970) and Rajini & Krishnakumari (1988), are typical of those resulting from cholinesterase inhibition, i.e. incontinence, salivation, chromolacrimation, tremors, fibrillations, fasciculations and prostration.

2.2 Short-term studies of toxicity

Rats

Groups of 12 male and 12 female rats (age 6–9 weeks) were fed diets containing pirimiphos-methyl (purity, 97%) at 0, 5, 8, 10 or 50 ppm (equivalent to 0, 0.5, 0.8, 1 and 5 mg/kg bw per day) for 28 days. Animals were checked daily for clinical signs. Food consumption (per cage of three rats) and body weight (per individual animal) were measured weekly. Plasma and erythrocyte cholinesterase activity were measured in groups of five males and five females on days −14, −7, 1, 3, 7, 14, 21 and 28. Brain cholinesterase was measured in groups of five males and five females on day 28. At termination on day 28, all animals were examined macroscopically.

There were no treatment-related clinical signs and pirimiphos-methyl had no effect on body-weight gain. Food consumption was slightly reduced (< 6%) in males at 5 ppm (statistically significant) and 8 ppm (not statistically significant). However, since food consumption was not affected in males at 10 and 50 ppm or in any of the groups of females, these findings were not considered to be toxicologically relevant. At termination, gross pathology did not reveal any lesions attributable to pirimiphos-methyl. Inhibition of plasma cholinesterase activity consistently exceeded 20% (up to 62%) in males and females at 50 ppm, at all days of treatment. Sporadic inhibition was noted at 8 and 10 ppm, as was sporadic elevation. However, erythrocyte cholinesterase activity was unaffected by administration of pirimiphos-methyl, even at 50 ppm. At termination, statistically significant inhibition of brain cholinesterase activity was observed at 50 ppm. However, the effects were small (11% in males, 13% in females) and, in the absence of concommitant clinical signs, were not considered to be adverse. Therefore, the no-observed-adverse-effect level (NOAEL) in this study was 50 ppm, equivalent to 5 mg/kg bw per day, i.e. the highest dose tested (Berry & Gore, 1975).

Groups of 30 male Wistar rats (age 8 weeks) were fed diets containing pirimiphos-methyl (purity, 90.5%) at a concentration of 0, 1000 or 1500 ppm (equivalent to 0, 100 and 150 mg/kg bw per day) for 28 days. Necropsy was performed on five rats per group at 7, 14, 21 or 28 days after initiation of exposure, and five rats per treated group were killed at 35 days (i.e. 7 days after withdrawal of pirimiphos-methyl). The fate of the remaining five rats was not reported. At each of the time-points, brain and erythrocyte acetylcholinesterase activity was measured. In addition, activity of plasma pseudocholinesterase and non-specific carboxylesterase in brain liver, plasma and kidney was assessed. Clinical signs and food consumption were recorded daily. Body weight was measured weekly.

It is reported that treatment with pirimiphos-methyl had no effect on clinical signs, food consumption, and body weight (data not shown). Brain and erythrocyte cholinesterase activity showed significant dose-related decreases at all time intervals during exposure (Table 2). Maximum reductions in brain cholinesterase activity were measured from days 14–28. Erythrocyte cholinesterase activity was consistently decreased from days 7–28. Post-exposure recovery of cholinesterase activity occurred in both treatment groups, but brain cholinesterase activity was still biologically significantly depressed (26 and 28% at 1000 ppm and 1500 ppm, respectively) 7 days after cessation of treatment. Plasma cholinesterase activity was variable, but was decreased by 17–44% over the various time intervals, the smallest reduction 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-points, but only after 14 days at 1000 ppm. Recovery was rapid and complete at both doses after withdrawal. Plasma non-specific carboxylesterase activity was markedly depressed at all time-points, but was still significantly depressed after 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.

Table 2. Brain and erythrocyte cholinesterase activity (% of control values) in rats given diets containing pirimiphos-methyl

Dietary concentration (ppm)	Cholinesterase	Treatment day				Post-treatment day
		7	14	21	28	7
1000	Brain	78	47	58	55	74
1500		65	39	44	46	72
1000	Erythrocyte	35	32	36	46	92
1500		32	30	25	33	82

From Rajini et al. (1989)

In this study a NOAEL could not be identified. The lowest-observed-adverse-effect level (LOAEL) was 1000 ppm, equivalent to 100 mg/kg bw per day (Rajini et al., 1989).

Five groups of 12 young male rats (strain unknown) were diets containing pirimiphos-methyl (purity, 90.5%) at a concentration of 0, 10, 250, 500 or 1000 ppm (equivalent to 0, 1, 25, 50 and 100 mg/kg bw per day) for 28 days. Animals were checked daily for clinical signs and mortality. Food consumption was measured daily, body weight was measured weekly. At termination blood was sampled by cardiac puncture. Brain, liver, lungs, heart, adrenals, kidneys, spleen and testes were weighed and examined histologically. Brain and plasma cholinesterase and liver and plasma enzyme activity were measured.

There were no effects on mortality, clinical signs, body-weight gain or food intake. Although compound intake was reported to be 0, 4, 100, 200 or 400 mg/rat, it was not indicated over what time-period this was consumed. Therefore, for the present evaluation the intake of compound is based on the standard 10 : 1 ppm to mg/kg bw per day conversion for young rats. 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 transaminase activity (at 1000 ppm) and increased alkaline phosphatase activity (at 500 and 1000 ppm) were noted. Hepatic transaminases (β-glucuronidase and alkaline phosphatase) were unaffected. Cholinesterase activity (plasma and brain) were dose-dependently inhibited at 250 ppm and above. At 250 and 500 ppm, brain cholinesterase activity was statistically significantly reduced by 18% and 27%, respectively. Based on the 27% reduction in brain cholinesterase activity at 500 ppm, the NOAEL was 250 ppm, equivalent to 25 mg/kg bw per day (Rajini & Krishnakumari, 1988).

Four groups of 25 male and 25 female Alderly Park SPF rats were fed diets containing pirimiphos-methyl (purity, 93.1%) at 0, 8, 80 or 360 ppm (equivalent to 0, 0.4, 4 and 18 mg/kg bw per day) for 90 days. Twenty rats of each sex per group were sacrificed at 90 days; the remaining animals were sacrificed after a 28-day recovery period. The animals were checked daily for clinical signs. Body weight and food consumption were measured weekly, haematology (haemoglobin, erythrocyte volume fraction, total and differential leukocyte counts, reticulocyte counts, mean cell haemoglobin concentration (MCHC), mean corpuscular diameter, platelets, clotting function tests) was performed for five rats of each sex per dose pre-test and after 6 and 13 weeks. Cholinesterase activity in plasma and erythrocytes was tested in five males and five females per group, five times pre-test and after 1, 2, 4, 6, 8, 10 and 12 weeks of dosing and after 1 and 4 weeks during the recovery period. At termination at 90 days, or after the 4-week recovery period, all animals were macroscopically examined, and absolute and relative organ weights (liver, heart, lung, adrenals, kidney, spleen) were assessed for five rats of each sex per group. Histopathology was performed on a selection (19) of organs. Brain cholinesterase activity was assessed in five rats of each sex per dose at 90 days and after the 4-week recovery period. In the report it is not indicated whether the observed effects reached statistical significance.

No treatment-related clinical signs were observed. Compared with controls, body-weight gain in females was reduced by 18% and 21% at 80 ppm and 360 ppm, respectively, but food intake in these groups was slightly increased. No effects were observed on haematological parameters. No effect of treatment on plasma and erythrocyte cholinesterase activity was observed 1 week after the start of dosing. Plasma cholinesterase activity was depressed in males (41–72%) and females (56–88%) during weeks 2–12 at 80 ppm and 360 ppm. Recovery to normal levels of activity was observed 1 week after withdrawal of pirimiphos-methyl. From weeks 2 to 12, erythrocyte cholinesterase activity was depressed in males (39–52%) and females (43–71%) at 360 ppm. In the group at 360 ppm, erythrocyte cholinesterase activity was inhibited by 34% in males and 29% in females 1 week after cessation of treatment. At week 4 of the recovery period, erythrocyte cholinesterase activity was

comparable to control levels. At the end of the treatment period, brain cholinesterase activity was depressed by 20% and 42% in females at 80 and 360 ppm, respectively. At the end of the 4-week recovery period, brain cholinesterase activity was still reduced by 21% and 35% in females at 80 and 360 ppm, respectively. Brain cholinesterase activity in males was not affected by treatment with pirimiphos-methyl. Histopathological examination revealed no treatment-related effects.

Based on the effects on body-weight gain and brain cholinesterase activity in females and on erythrocyte cholinesterase activity in both sexes at 80 ppm, the NOAEL was 8 ppm, equivalent to 0.4 mg/kg bw per day (Clapp & Conning, 1970).

Dogs

Groups of four male and four female beagle dogs were fed gelatin capsules containing pirimiphos-methyl (purity unknown) at a dose of 0, 2, 10 or 25 mg/kg bw per day for 3 months. Animals were checked daily for mortality and clinical signs. Food consumption was recorded twice per day. Body weight was assessed weekly. Ophthalmoscopy was performed before dosing and at weeks 6 and 12. Plasma and erythrocyte cholinesterase activity were assessed five times before dosing, and after 1, 2, 4, 6, 8, 10 and 12 weeks. Haematology, clinical chemistry and urine analysis was performed before dosing and at weeks 6 and 12. Urine analysis and electrocardiography were performed before dosing and at week 12. After 3 months, 2 animals of each sex per group were killed. The remaining animals were allowed to recover for 4 weeks and then killed. All animals were examined macroscopically and a range of organs were weighed and examined histologically. A sample of the left frontal cortex was taken for assessment of cholinesterase activity.

There were no mortalities. In the group at the highest dose, increased incidences of vomiting and loose stools were observed. Furthermore, these animals showed a marked loss of general condition (dry skin, dull coat). In this group, body-weight gain and food consumption were reduced throughout the treatment period. At week 12, a significant reduction in heart rate was found. In the groups at 2 and 10 mg/kg bw per day, body weight gain was similar to that of controls, except for one dog in the group at the intermediate dose, which showed a reduction in body-weight gain from week 6 onwards. Water consumption was not affected. Ophthalmoscopy revealed no effects on the eyes. No consistent effects on haematology, clinical chemistry and urine analysis were observed.

Compared with the values for concurrent controls, erythrocyte cholinesterase activity was consistently and dose-dependently reduced (> 20%) at 10 and 25 mg/kg bw per day, at all time-points after the start of the treatment. Maximum levels of erythrocyte cholinesterase inhibition were reached from weeks 2–4 onwards. Treatment had no effect on brain cholinesterase activity. On the basis of inhibition of erythrocyte cholinesterase activity, the NOAEL was 2 mg/kg bw per day (Noel et al., 1970).

2.3 Reproductive toxicity: developmental toxicity

Rabbits

Groups of 16 female New Zealand White rabbits were given pirimiphos-methyl (purity, 98.8%) at a dose of 0, 12, 24 or 48 mg/kg bw per day (vehicle, corn oil) by gavage once per day during days 6–18 of gestation (day of insemination was denoted day 0). Clinical signs and mortality were examined daily, with particular attention being paid to the 1–2 h after dosing. Body weight and body-weight gain were determined on days 4, 6–19, 22, 26 and 29 of gestation. Food consumption was measured daily. Blood samples were taken from six animals of each group for plasma and erythrocyte cholinesterase analysis on days 5, 19 and 29 of gestation. Time of blood sampling was not specified. At termination on day 29 of gestation, the females were necropsied and ovaries and uterus were examined for number of corpora lutea, implantation sites, live and dead pups and early and late

resorptions. A sample of brain (from the frontal cortex) of approximately 0.1 g was removed from the same six animals in each group and examined for brain cholinesterase activity. Fetuses were weighed, sexed and examined for external, internal and skeletal abnormalities and anomalies. Statements of adherence to quality assurance and GLP were included.

No treatment-related deaths or toxicologically relevant clinical effects were observed. Body weight and food consumption were not significantly affected. The effects of treatment on plasma, erythrocyte and brain cholinesterase activity are described in Table 3.

Table 3. Plasma, erythrocyte and brain cholinesterase activity (% of control values) in rabbits given pirimiphos-methyl by gavage

Time-point	Cholinesterase	Dose (mg/kg bw per day)		
		12	24	48
Day 5	Plasma	102	93	92
	Erythrocyte	98	110	107
Day 19	Plasma	87	63*	50*
	Erythrocyte	79	56*	38*
Day 29	Plasma	102	94	106
	Erythrocyte	110	91	82
	Brain	142	160	62*

From Barton & Hastings (1994).
* $p < 0.05$, statistically significant

At the intermediate and highest doses, maternal toxicity was indicated by depressed erythrocyte cholinesterase activity on day 19. In the group at the highest dose, brain cholinesterase activity was decreased at termination. No toxicologically relevant effects were observed in dams in groups at the lowest dose. On the basis of reduced erythrocyte cholinesterase activity at day 19 in dams at the intermediate dose, the NOAEL for maternal toxicity was 12 mg/kg bw per day.

Pirimiphos-methyl did not induce irreversible structural changes and had no toxicologically relevant effects in fetuses. Therefore the NOAEL for embryo/fetotoxicity was 48 mg/kg bw per day, i.e. the highest dose tested (Barton & Hastings, 1994).

2.4 Special studies: neurotoxicity

Rats

A group of 25 male rats (strain unknown) received a single dose (method of administration not specified) of pirimiphos-methyl (purity, 90.5%) at 1000 mg/kg bw. An additional group of 25 rats served as controls. Five rats per group were sacrificed at 4, 8, 24, 48 or 72 h after dosing and plasma and brain cholinesterase and non-specific carboxylesterase activities were measured. Plasma cholinesterase inhibition was rapid (60% inhibition by 4 h), while inhibition of brain cholinesterase activity was slower (36% by 8 h). Both attained maximum inhibition by 24 h (93% for plasma and 61% for brain). Partial recovery was apparent at 48–72 h for both enzymes, but that for brain was slower. Inhibition of non-specific carboxylesterase activity attained maximum levels (plasma, 80%; and brain, 47%) at 24 h; compared with cholinesterase, inhibition was slightly less, and recovery in plasma was more rapid. In brain, recovery of non-specific carboxylesterase and cholinesterase activity were comparable (Rajini & Krishnakumari, 1988).

Groups of 17 male and 17 female Sprague-Dawley rats received a single oral (gavage) dose of pirimiphos-methyl at 0, 15, 150 or 1500 mg/kg bw in corn oil. In each group, seven animals of each sex were used for neuropathology analysis and ten animals of each sex were allocated for cholinesterase evaluation. Animals were checked daily for viability and clinical signs. Body weight was measured before the test and on days 0, 1, 7, 14 and 15 of treatment. A functional observation battery (FOB) test and a locomotor activity test were performed before the test, at the time of peak effect (± 24 h after dosing—study day 1) and on days 7 and 14 for the seven animals of each sex per group used for neuropathology evaluation and for five animals of each sex per group used for cholinesterase evaluation. In the animals used for cholinesterase evaluation, plasma and erythrocyte cholinesterase activity was determined in five animals of each sex before initiation of dosing, at the time of peak effect (± 24 h after dosing), and on days 7 and 15. Brain cholinesterase activity was assessed in five animals of each sex per group at the time of peak effect (day 1) and at day 15. Blood was collected at euthanization and whole brain weights and regional brain weights were recorded for each animal. In the neuropathology group, all animals were euthanized on day 15 and perfused in situ. A neurohistopathological examination was performed for five animals of each sex in the control group and in the group at 1500 mg/kg bw. Statements of adherence to quality assurance and GLP were included.

No mortality was observed. In animals at 1500 mg/kg bw, clinical signs typical of cholinesterase inhibition were observed, i.e. tremors, lacrimation, staining on body surface, gait alterations, hunched behaviour, exophthalmus, soft stools. These signs were predominantly observed on day 1, although in some animals clinical signs were also observed on days 2–4. In the group at the highest dose, FOB testing on day 1 revealed altered posture (sitting with head lowered, flattened), clonic convulsions (whole body tremors), altered palpebral closure (eyelids drooping or half-closed), lacrimation, salivation, soiled fur, red deposits around eyes, nose and mouth and chromodacryorrhea, catalepsy, altered pupil response and righting reflex, decrease in body temperature, altered hindlimb extensor strength and reduced forelimb and hindlimb grip strength and a reduced rotarod performance. Also, in the group at the highest dose, the motor activity test on day 1 revealed decreased motor activity, gait alterations (walking on tiptoes, hunched body, ataxia), clonic convulsions (whole body tremors; slight or moderate), and decreased arousal and rearing activity. Loss of body weight in the group at the highest dose was observed on the first day of testing. During the next 2 weeks, body weights recovered to control levels. No clinical signs and no effects on FOB or locomotor activity parameters were observed in animals at 15 and 150 mg/kg bw. Treatment with pirimiphos-methyl had no effect on total brain weight, regional brain weight or brain histology in any of the treatment groups.

The effects of treatment with pirimiphos-methyl on cholinesterase activity on day 1 and day 15 are presented in Table 4.

Table 4. Cholinesterase activity (% of control values) in rats given a single oral dose of pirimiphos-methyl by gavage

Effect	Dose (mg/kg bw)					
	15		150		1500	
	Male	Female	Male	Female	Male	Female
Day 1						
Plasma	79*	52*	45*	19*	8*	4*
Erythrocyte	74*	95	61*	79*	29*	37*
Brain sections:						
Hippocampus	97	97	91	86*	35*	42*
Olfactory	91	93	88	94	36*	42*

Midbrain	90*	97	77*	83*	32*	41*
Brainstem	96	98	82*	84*	33*	41*
Cerebellum	93	96	71*	72*	28*	35*
Cortex	100	102	91*	87*	36*	43*
Day 15						
Plasma	119	82	108	101	115	85
Erythrocyte	98	112	88*	97	85*	92
Brain sections:						
Hippocampus	98	91	107	103	76	69
Olfactory	99	90	94	91	79	66*
Midbrain	/3	83	89	78	73*	69*
Brainstem	102	101	95	96	86*	86*
Cerebellum	101	98	105	107	90	91
Cortex	103	100	99	92	71*	70*

From Nemec (1995)
* $p < 0.05$, statistically significant

At day 1, dose-dependent reductions in plasma, erythrocyte and brain cholinesterase activity were observed. At day 15, inhibition of plasma and erythrocyte cholinesterase was < 20% for all treated groups. Regional brain cholinesterase activity was affected in a dose-related manner in the two groups at higher doses. At day 1, some brain regions showed a (significant) inhibition of brain cholinesterase activity of > 20% at the intermediate dose and in all brain regions in the highest dose. At day 15, inhibition of cholinesterase activity of > 20% was observed only in the midbrain of females at the intermediate dose and in most brain regions at the highest dose.

On the basis of inhibition of brain cholinesterase activity in some brain regions at 150 mg/kg bw, the NOAEL was 15 mg/kg bw (Nemec, 1995).

3. Observations in humans

Five healthy men (body weight, 59.5–73 kg bw; age 25 45 years) were given pirimiphos-methyl (purity, 97.8%) at a dose of 0.25 mg/kg bw per day orally for 28 days. Blood samples for measurement of plasma and erythrocyte cholinesterase activity were taken on days −14, −7, 1, 3, 7, 14, 21 and 28.

One subject showed inhibition of plasma cholinesterase activity (21.5%) on day 28. Otherwise changes in cholinesterase activity, both above and below values measured before dosing, were within 12%. Four of five subjects had erythrocyte cholinesterase activity values that were 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 men (body weight, 62–73 kg; age 22–27 years) and four women (body weight, 44–60 kg; age 21–49 years) were given capsules containing pirimiphos-methyl (purity, 97.8%) at a dose of 0.25 mg/kg bw per day for 56 days. Blood samples, for measurement of plasma and erythrocyte cholinesterase activity, liver enzymes and haematology, were taken twice before initiation of dosing, and on days 7, 14, 21, 28, 35, 42, 49 and 56 of the study, and also during the recovery period 7, 14, 21 and 29 days after treatment. Controls comprised two women (body weight, 44 and 46 kg; age 29 and 30 years).

No compound-related effects were observed on liver function (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase or glutanyl transpeptidase activities in plasma), haematology (haemoglobin, erythrocyte volume fraction, MCHC, total and differential leukocyte counts, platelets, erythrocyte sedimentation rate) or erythrocyte cholinesterase activity. Plasma cholinesterase was depressed about 20% in two of four women on days 14, 21 and 28 and in one woman on days 28 and 35. The effect did not increase with time. All values were normal during the withdrawal period (Howard & Gore, 1976).

Comments

Biochemical aspects

No new toxicokinetic studies were available for the present evaluation. The evaluation made by the 1992 JMPR indicated that peak plasma concentrations of radioactivity (after administration of [C^{14}]pirimiphos-methyl) are reached 0.5 h after an oral dose. Pirimiphos-methyl is rapidly excreted. After oral administration of pirimiphos-methyl to male rats, 80.7% and 7.3% of the administered dose was excreted via the urine and faeces, respectively, within 24 h. In dogs, 48 h after dosing at either 18.4 or 16.7 mg/kg bw, urinary excretion was 64.4% or 82.5% and faecal excretion was 17.3% or 13.3%, respectively.

As a thiophosphate, pirimiphos-methyl requires metabolic activation (from P=S to P=O) to inhibit acetylcholinesterase activity. No data were available on the interindividual variability of P=S oxidation. Pirimiphos-methyl is highly lipophilic (log K_{ow} = 4.2).

Toxicological data

The acute oral toxicity of pirimiphos-methyl is low. In the rat, acute oral median lethal dose (LD_{50}) values ranging from 1667 to 2050 mg/kg bw. The clinical signs observed in the LD_{50} experiments are typical of those resulting from inhibition of acetylcholinesterase activity, i.e. incontinence, salivation, chromolacrimation, tremors, fibrillations, fasciculations and prostration.

A number of 28-day and 90-day studies with pirimiphos-methyl were performed in rats and dogs. In all these studies, inhibition of cholinesterase activity was the critical end-point. The overall NOAEL from the studies in rats was 8 ppm, equivalent to 0.4 mg/kg bw per day. The NOAEL in a 90-day study in dogs was 2 mg/kg bw per day. There were no indications that dogs are more sensitive than rats to the effects of pirimiphos-methyl. In these short-term studies it appeared that inhibition of erythrocyte cholinesterase activity reached maximum levels only after 2 weeks of treatment.

In a study of developmental toxicity in rabbits, the NOAEL for maternal toxicity was 12 mg/kg bw per day on the basis of a reduction in erythrocyte acetylcholinesterase activity on day 19 at 24 mg/kg bw per day. No toxicologically relevant effects in fetuses were observed. The NOAEL for embryo-fetotoxicity was 48 mg/kg bw per day. In dams treated at 48 mg/kg bw per day, brain cholinesterase activity was still significantly inhibited at day 29, i.e. 11 days after the last dose.

Two single-dose studies of neurotoxicity in rats were available. In the first, after administration of a high dose (1000 mg/kg bw) of pirimiphos-methyl, maximum inhibition (61%) of brain acetylcholinesterase activity was found after 24 h. Partial recovery was apparent at 48–72 h. In the second single-dose study of neurotoxicity, rats treated with pirimiphos-methyl at 150 or 1500 mg/kg bw showed dose-dependent reductions in erythrocyte and brain acetylcholinesterase activity 24 h after administration. In the animals at the highest dose, brain acetylcholinesterase activity had only partially recovered by day 15 after treatment. On the basis of the inhibition in brain cholinesterase activity at 24 h, the NOAEL was 15 mg/kg bw.

In one 28-day and one 56-day study in humans, pirimiphos-methyl was administered orally at a dose of 0.25 mg/kg bw per day. In neither study was inhibition of erythrocyte acetylcholinesterase activity nor any other toxicologically relevant effect observed.

Toxicological evaluation

The critical effect caused by pirimiphos-methyl is inhibition of acetylcholinesterase activity in the nervous system. Pirimiphos-methyl is not embryo-fetotoxic.

In establishing an ARfD, the Meeting concluded that it is appropriate to use data on inhibition of acetylcholinesterase activity in rats from a single-dose study of neurotoxicity in which a NOAEL of 15 mg/kg bw was identified. Based on this NOAEL, the Meeting established an ARfD of 0.2 mg/kg bw, using a safety factor of 100.

The Meeting considered that it was not appropriate to use a chemical specific adjustment factor, although the occurrence and severity of the adverse effects of acetylcholinesterase inhibitors (directly related to the level of inhibition of cholinesterase activity in the nervous system) are considered to depend on C_{max} rather than the area under the curve. In fact, the Meeting observed that:

- Peak plasma concentrations of radioactivity (after administration of ^{14}C-labelled pirimiphos-methyl) are reached 0.5 h after an oral dose, while maximal inhibition of brain acetylcholinesterase activity appears to occur after about 24 h.
- Pirimiphos-methyl is highly lipophilic (log K_{ow} = 4.2). As a thiophosphate, it requires metabolic activation (from P=S to P=O) to inhibit acetylcholinesterase activity. No data are available on the interindividual variability of P=S oxidation.
- The recovery of brain cholinesterase activity is slow.

Levels relevant for risk assessment

Species	Study	Effect	NOAEL	LOAEL
Rat	Acute neurotoxicity[a]	Neurotoxicity	15 mg/kg bw	150 mg/kg bw
Human	28-day, 56-day toxicity	(Neuro-)toxicity	0.25 mg/kg bw	—[b]

[a] Gavage administration
[b] Highest dose tested

Estimate of acute reference dose

0.2 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

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