

MALATHION (addendum)

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Explanation

Malathion was evaluated by the JMPR in 1963, 1965 and 1966 (Annex 1, references 2, 3 4, and 6). An acceptable daily intake (ADI) of 0–0.02 mg/kg bw was assigned at each Meeting. Malathion was re-evaluated by the JMPR in 1997 (Annex 1, references 80 and 81), when an ADI of 0–0.3 mg/kg bw was assigned. Malathion was re-evaluated at the present Meeting in order to establish an acute reference dose (RfD), at the request of the Codex Committee on Pesticide Residues. The Meeting reviewed a study in humans, some studies of toxicity in animals, and studies of genotoxicity, which had been produced since the last evaluation by the JMPR. The FAO and WHO were in the process of revising the specifications for malathion technical material.

Evaluation for acute reference dose**1. Effects on enzymes and other biochemical parameters**

A study was undertaken by Fulcher et al. (2001) to investigate the effects of orally-administered malathion (purity, 96%) on cholinesterase activity in Crl:CD®BR rats. In this study, which was a supplement to a study of developmental neurotoxicity (see below, and Fulcher, 2003), pregnant rats, offspring at various stages of development before weaning, and young adults (aged 7–8 weeks) were given single or repeated doses of malathion of up to 450 mg/kg bw. Groups of nine mated female animals were given malathion at a daily dose of 0, 5, 50 or 150 mg/kg bw by gavage on days 6–20 of gestation. Eight rats per group were killed 3 h after the last dose, litter data were assessed and maternal and fetal plasma, erythrocyte and brain cholinesterase activities were determined. Additional groups of 10 mated females were treated with the same daily doses from day 6 of gestation until postnatal day 10. These dams were allowed to litter and rear their young until weaning on postnatal day 21, the litters being culled to four males and four females each on postnatal day 4, when measurements of plasma, erythrocyte and brain cholinesterase activity were made for selected culled offspring from each litter. The remaining offspring from eight litters per

group were then given malathion during postnatal days 11–21, in order to assess effects on survival, body-weight gain and plasma, erythrocyte and brain cholinesterase activities; selected offspring were killed on postnatal day 21 or 60. Another group of eight untreated pregnant females was allowed to give birth to litters and to rear their young. The litters were culled to five pups of each sex on postnatal day 4. On postnatal day 11, one male and one female per litter were assigned to each group (eight pups of each sex per group) and given a single dose of malathion of 0, 5, 50, 150 or 450 mg/kg bw. These pups were killed 2 h after dosing, and plasma, erythrocyte and brain cholinesterase activities were determined. Additionally, young adult animals (eight of each sex; aged 7–8 weeks) were given malathion at a dose of 0, 5, 50, 150 and 450 mg/kg on one occasion, and were killed 2 h later and plasma, erythrocyte and brain cholinesterase activities were determined. Determination of plasma, erythrocyte and brain cholinesterase activities was carried out for eight male and eight female young adults that were given malathion at a dose of 0, 5, 50 or 150 mg/kg bw per day for 11 days and killed 2 h after the last dose. Determination of cholinesterase activity was undertaken using a modified Ellman method (Environmental Protection Agency, 1996). Because of the complicated study design, the findings are discussed for all groups (i.e. pregnant rats, offspring at various stages of development and young adults) by dose. In all cases, inhibition of cholinesterase activity is expressed as group mean reduction (%) relative to values for the appropriate concurrent control group.

450 mg/kg bw per day

Among offspring of untreated dams given a single dose of malathion of 450 mg/kg bw on postnatal day 11, body tremors were observed 1–2 h after dosing in five out of 16 animals and an additional pup was killed in extremis. Marked inhibition of plasma, erythrocyte and brain cholinesterase activities was seen, with inhibitions of 54%, 72% and 84% in plasma, erythrocytes and brains of males, respectively, and inhibitions of 52%, 61% and 81% in plasma, erythrocytes and brains of females, respectively. In young adults given a single dose of 450 mg/kg bw, plasma and erythrocyte cholinesterase activities were inhibited by 24% and 25% in males, respectively, and by 11% and 17% in females, respectively. Brain acetylcholinesterase activity was not inhibited in animals of either sex.

150 mg/kg bw

No adverse effects were seen in dams or young adult rats given repeated doses of malathion at 150 mg/kg bw per day, as assessed by clinical signs, body weight and body-weight gain, including during pregnancy and lactation and at necropsy. Litter data on day 20 of gestation and on postnatal day 11, fetal weight on day 20 of gestation, pup weight on postnatal day 1 and pup body-weight gain until postnatal day 11 were unaffected by treatment of the dams. In pups (taken from dams treated from day 6 of gestation until postnatal day 10) treated with malathion at a dose of 150 mg/kg bw from postnatal day 11 until weaning on postnatal day 21, no adverse effects were observed as assessed by clinical signs, survival or body-weight gain through to postnatal day 60. On day 20 of gestation, the dams treated with malathion exhibited depression of erythrocyte cholinesterase activity (inhibition of 51% compared with controls), but there was no inhibition of plasma or brain cholinesterase activity. Fetal plasma and erythrocyte cholinesterase activities were inhibited by 15% and 19%, respectively, on day 20 of gestation, while the activity of brain cholinesterase was unaffected. On postnatal day 4, cholinesterase activity in pups was comparable in test and control groups. Direct treatment of offspring of untreated dams was associated with marked inhibition of plasma, erythrocyte and brain cholinesterase activities on

postnatal day 11: plasma cholinesterase activity was inhibited by 36% in males and 35% in females; erythrocyte cholinesterase activity was inhibited by 55% in males and 48% in females; and brain cholinesterase activity was inhibited by 44% in males and 48% in females. For young adults, a single dose of 150 mg/kgbw had no significant effect on cholinesterase activity in plasma, erythrocytes or brain. Direct treatment of the offspring of treated dams with 11 doses administered during postnatal days 11–21 caused inhibition of plasma, erythrocyte and brain cholinesterase activities; inhibition in erythrocytes was marked. Plasma cholinesterase activity was inhibited by 24% in males and 32% in females, erythrocyte cholinesterase activity was inhibited by 67% in males and 68% in females and brain cholinesterase activity was inhibited by 16% in both sexes. Young adult males treated for 11 days showed inhibition of plasma and erythrocyte cholinesterase activities of 13% and 43%, respectively, while brain cholinesterase activity was unaffected. Young female adults treated for 11 days showed inhibition of plasma and erythrocyte cholinesterase activities of 13% and 48%, respectively, while the activity of brain cholinesterase was not affected. On postnatal day 60 (39 days after the end of treatment), offspring showed no discernible treatment-related effect on the activity of erythrocyte or brain cholinesterase, but plasma cholinesterase activity was inhibited by 13% and 23% in males and females, respectively.

50 mg/kg bw

No adverse effects were seen in dams or young adults rats given malathion at a dose of 50 mg/kgbw per day, as assessed by clinical signs, body weight and body-weight gain, including during pregnancy and lactation and at necropsy. Litter data on day 20 of gestation and postnatal day 11, fetal weight on day 20 of gestation, pup weight on postnatal day 1 and pup body-weight gain until postnatal day 11 were unaffected by treatment of the dams. Treatment at this dose had no effect on pups from postnatal day 11 until weaning on postnatal day 21, as assessed by clinical signs, survival or body-weight gain through to postnatal day 60. On day 20 of gestation, the dams showed marginal inhibition of erythrocyte cholinesterase activity (19%), but neither plasma nor brain cholinesterase activities were inhibited. Fetal plasma and erythrocyte cholinesterase activities were marginally inhibited on day 20 of gestation (14% and 11% respectively), while brain cholinesterase activity was unaffected. On postnatal day 4, plasma, erythrocyte and brain cholinesterase activities in the pups were comparable in treated and control groups. At postnatal day 11, treatment of offspring of untreated dams was associated with inhibition of plasma and erythrocyte cholinesterase activities (inhibitions of 19% and 25% in males, and 16% and 23% in females, respectively), while brain cholinesterase activity was only slightly affected (inhibition of 6% and 10% in males and females, respectively). For young adults, treatment with a single dose of malathion had no significant effect on the activity of cholinesterase in plasma, erythrocytes or brain. Treatment of the offspring during postnatal days 11–21 caused inhibition of plasma and erythrocyte cholinesterase activities, inhibition being 19% and 39% in males and 19% and 34% in females, respectively. Cholinesterase activity in the brain was unaffected. Young adults treated with malathion for 11 days showed a minor degree of inhibition of plasma cholinesterase activity (11% in males and 15% in females), while erythrocyte cholinesterase activity was inhibited by 20% in both sexes. Activity of the brain enzyme was not inhibited. On postnatal day 60 (39 days after the end of treatment), there was no discernible effect on the erythrocyte or brain cholinesterase activity in offspring, while the plasma cholinesterase activity was inhibited by 5% and 16% in males and females, respectively.

5 mg/kg bw

At 5 mg/kg bw per day, there were no adverse effects in dams and young adult rats as assessed by clinical signs, body weight and body-weight gain, including during pregnancy and lactation and at necropsy. Litter data on day 20 of gestation and postnatal day 11, fetal weight on day 20 of gestation, pup body weight on postnatal day 1 and pup body-weight gain through to postnatal day 11 were not affected by treatment given to the dams. No effect of treatment of pups from postnatal day 11 to weaning on postnatal day 21 was observed in respect of clinical signs, survival or body-weight gain through to postnatal day 60. On day 20 of gestation, the dams did not show inhibition of erythrocyte or brain cholinesterase activity, although plasma cholinesterase activity was inhibited by 12%. Fetal plasma, erythrocyte and brain cholinesterase activities were unaffected by treatment. On postnatal day 4, cholinesterase activity in pups was comparable in the test groups and the controls. Treatment of offspring of untreated dams on postnatal day 11 did not affect plasma, or brain cholinesterase activities, but erythrocyte cholinesterase activity was observed to be marginally (16%) inhibited. For young adults, treatment with a single dose of malathion had no significant effect on cholinesterase activity in plasma, erythrocytes or brain. Treatment of the offspring during postnatal days 11–21 produced only minor degrees of inhibition of plasma (13% and 10% in males and females, respectively) and erythrocyte (17% and 15% in males and females, respectively) cholinesterase activity. The activity of brain cholinesterase was unaffected. Young adults treated for 11 days showed no inhibition of plasma, erythrocytic or brain cholinesterase activity. On postnatal day 60 (39 days after the end of treatment), offspring showed no discernible effect on cholinesterase activity, except for plasma cholinesterase in females, which was inhibited by 18%. The no-observed-adverse-effect level (NOAEL) for the study was 50 mg/kg bw. Inhibition of brain cholinesterase activity was observed in offspring of untreated females after direct treatment with a single dose of malathion of 150 mg/kg bw on postnatal day 11. In this study, erythrocyte cholinesterase activity was more sensitive to inhibition induced by malathion than was brain cholinesterase activity (Fulcher et al., 2001).

2. Toxicological studies

2.1 Genotoxicity

Table 1 summarizes the results of studies of genotoxicity with malathion.

Table 1. Results of studies of genotoxicity with malathion

End-point	Test object	Concentration/dose	Purity	Results	Reference
<i>In vitro</i>					
Chromosomal aberration	Human lymphocytes	12.5–800 µg/ml –S9, 75–1800 µg/ml +S9	96%	Positive –S9 (at toxic concentrations)	Edwards (2001a) ^a
Gene mutation	Mouse lymphoma cells (L5178Y)	125–2000 µg/ml –S9, 250–2200 µg/ml –S9	96%	Positive ± S9 (at markedly toxic concentrations)	Edwards (2001b) ^b
<i>In vivo</i>					
Unscheduled DNA synthesis	Male Wistar rats	500–2000 mg/kg bw (by gavage)	96%	Negative	Meerts & van de Waart (2003) ^c

S9, 9000 × g supernatant fraction from rodent liver

^a Test complied with GLP (United States Food and Drug Administration, 21CFR58; Japanese Ministry of Health and Welfare, PAB 414; European Commission, 1999/11/EC) and with OECD Guideline 473

^b Test complied with GLP (United Kingdom SI 3106; OECD ENV/MC/CHEM(98)17; and European Commission 1999/11/EC)

^c Test complied with GLP (United States Food and Drug Administration, 21CFR58; United States Environmental Protection Agency, 40CFR160, 40CFR792) and with OECD Guideline 486

2.2 *Special studies*

(a) *Teratogenicity*

A study of teratogenicity in rabbits, evaluated by the 1997 JMPR, was recently re-evaluated by Robinson (2002), and is briefly described again here. Subsequent to a range-finding study, malathion (purity, 92.4%) in corn oil was administered at a dose of 25, 50 and 100 mg/kg bw per day by gavage to groups of 20 mated New Zealand white female rabbits during days 6–18 of gestation; controls received corn oil only. Although there were no statistically significant differences in survival between the treated and control groups, no deaths occurred among the controls, while there were four deaths in the group receiving the lowest dose, three in the group receiving the intermediate dose and two in the group receiving the highest dose. Both instances of mortality in the group receiving the highest dose resulted from accidental intrapulmonary intubation during dosing. No clear cause of death was established for mortality occurring at the two lower doses. The study authors concluded that there was no dose–response relationship with regard to mortality. There was a decrease in maternal body-weight gain during days 6–18 of gestation at a dose of 50 and 100 mg/kg bw per day. On days 12, 18 and 29, the mean body weight of the group receiving the highest dose was lower than that of the controls. Therefore, the NOAEL for maternal toxicity was 25 mg/kg bw per day. There was a slight increase in the mean number and per cent of resorptions at ≥ 50 mg/kg bw per day. There was no difference in fertility, number of corpora lutea, implantation sites, litter size or fetal weight and length. No other signs of toxicity were seen in does or fetuses, nor was there any evidence for teratogenicity. The 1997 Meeting considered that the NOAEL was 25 mg/kg bw per day for maternal toxicity, on the basis of decreased body-weight gain at the intermediate dose, and 100 mg/kg bw per day for fetal toxicity, on the basis of the failure to observe fetal toxicity at any dose (Siglin, 1985). The embryoletality observed in this study, and in the range-finding study was re-evaluated by Robinson (2002). In the re-evaluation, the data for animals treated at a dose of 25, 50 or 100 mg/kg bw per day were combined from these two studies; it was concluded that treatment with malathion had no effect on postimplantation loss (or any other parameter for development). Thus, the NOAEL of 100 mg/kg bw per day for fetal toxicity was maintained.

(b) *Developmental neurotoxicity*

A study of developmental neurotoxicity with orally administered malathion (purity, 96%) in CrI:CD@BR rats was undertaken by Fulcher et al. (2002) in compliance with GLP (United Kingdom SI 3106, OECD, ENV/MC/CHEM(98)17, European Commission, 1999/11/EC) and with United States Environmental Protection Agency (EPA) guideline subdivision F, OPPTS 870.6300.

Malathion was administered to groups of at least 21 mated females on day 6 of gestation until postnatal day 10, and to their offspring on postnatal days 11–21 at a dose of 5, 50 or 150 mg/kg bw per day; a control group received the vehicle, namely corn oil. The F₀ dams were inspected twice daily. Body weights were recorded on days 0, 3, 6, 10, 14, 17 and 20 of gestation and thereafter daily until parturition. The dams were also weighed on postnatal days 1, 4, 11, 14, 17 and 21. Food consumption was recorded for the following periods: days 0–2, 3–5, 6–9, 10–13, 14–16 and 17–19 of gestation and postnatal days 1–3, 4–6, 7–10, 11–13, 14–16 and 17–20. Behavioural assessments were performed on at least 10 dams per group. These comprised arena and in-hand observations on days 12 and 18 of gestation and postnatal days 4 and 10. Arena observations comprised degree of eyelid

closure, posture, gait, tremor, twitches and convulsions or absence thereof, activity counts, rearing counts, grooming, urination and defecation. In-hand observations comprised ease of removal from cage, salivation, lacrimation, piloerection, exophthalmos, pupil closure reflex, condition of fur and reactivity to handling. All offspring were examined 24 h after birth; the number of live and dead offspring and their body weights and sex were recorded, and they were observed clinically. A daily record was maintained of mortality in pups from birth until postnatal day 21. On postnatal day 4, the litters were culled to eight offspring per litter; offspring from each litter were allocated for behavioural assessment. Offspring were weighed on postnatal days 1, 4, 7, 11–21 and 28, and weekly thereafter. On postnatal day 11, the litters were culled to seven animals per litter to provide a male or female pup for necropsy, while on postnatal day 21 litters were culled to six animals, to provide one additional pup per litter for necropsy. If possible, this pup was of the opposite sex to that culled on postnatal day 11. Pups were inspected twice daily for signs of ill health. Behavioural observations on the pups were carried out as follows: arena observations were carried out on postnatal days 4, 11, 21, 35, 45 and 60, in-hand observations on postnatal days 35, 45 and 60, assessment of motor activity on postnatal days 13, 17, 22 and 59, assessment of auditory startle response inhibition on postnatal days 23/24 and 60/61, assessment of auditory startle pre-pulse inhibition on postnatal days 23/24 and 60/61, and assessment of learning and memory (Morris maze) on postnatal days 23/24 and 61/62. Sexual maturation was ascertained by examination for balano-preputial separation in males from postnatal day 38 onwards and by examination for vaginal opening in females from postnatal day 28 onwards. On postnatal day 11, one pup per litter was killed and 10 pups of each sex were perfused for neuropathological examination. Dams and one pup per litter were killed after weaning at postnatal day 21, with 10 male and 10 female pups being perfused with fixative for neuropathological examination. The remainder of the offspring were killed on postnatal days 63–67 and 10 males and 10 females were perfused with fixative for neuropathological examination of the brain. Additionally, selected organ weights were recorded for one male and one female per litter. Treatment of the dams at any dose had no effect on survival, clinical condition, body-weight gain or food intake, either during gestation or lactation. No effects related to treatment with the test material were observed on length of gestation, and parturition was normal; moreover, there were no effects on the results of behavioural assessments made on day 12 or 18 of gestation or postnatal day 4 or 10. Salivation after dosing was seen at all doses, including the controls. This effect, which was most severe at the highest dose, was considered to be a consequence of distaste for the formulation. Absolute and relative weights of reproductive organs and the brain did not differ between groups. Implantation counts, litter size on postnatal day 1 and survival to weaning (postnatal day 21) were similar in all groups. Body-weight gain of offspring in all groups was similar. In the offspring, most behavioural examinations showed little of note. During direct treatment of the offspring (postnatal days 11–21), tremors and underactivity were seen in four offspring from one litter, at 150 mg/kg bw; the effect was seen on days 7, 8 and 9 of treatment. This was thought to be a direct effect of treatment and not evidence for developmental neurotoxicity. On postnatal day 11, however, five female pups at 150 mg/kg bw per day failed to show an immediate surface righting reflex (only one pup had a slow surface righting reflex in the control group). At subsequent times (on postnatal day 22 in male pups and on postnatal day 59 in pups of both sexes), motor activity was not affected by treatment. On postnatal day 22, rearing and cage-floor activity was lower in treated females than in the controls, some differences being significant; however, this was not corroborated by an increase in time to complete the Morris water maze. Also there was considerable variation within the groups. On postnatal day 23/24, group mean startle amplitudes for treated offspring during startle habituation and during pre-pulse inhibition were higher than those of controls, and some of these

differences were significant. However, no dose–response relationship was observed. No such findings were elicited on postnatal day 61/62. At examination of the dams (postnatal day 21) and the pups (postnatal days 11, 21, 63–67) post mortem, no histopathological changes of significance were found. There were no effects of the treatment on the weights of the reproductive organs of dams (postnatal day 21) or offspring (postnatal day 63–67). There were no effects on brain weight, length and width among the pups on postnatal days 11, 21 and 63–67. Neuropathological examination of the offspring on postnatal day 21 and 63–67 did not reveal any findings that were related to treatment. The study authors considered that the NOAEL for the study was 50 mg/kg bw per day for developmental neurotoxicity (slower surface righting reflex at the highest dose of 150 mg/kg bw per day) and 150 mg/kg bw per day for maternal toxicity. The present reviewer agreed with this assessment and noted that the effects observed were likely to be caused by current treatment rather than any permanent developmental neurotoxic effect.

3. Observations in humans

A randomized double-blind placebo-controlled ascending single dose study was carried out in humans treated orally with malathion; the study complied with good clinical practice (GCP) (CPMP/ICH/135/95) and GLP (OECD, USEPA 40 CFR 160). Forty-eight healthy men and women (aged 18–50 years) were given gelatin capsules containing malathion (purity, 95.8%) at a dose of 0.5, 1.5, 5, 10 or 15 mg/kg bw, while controls received a placebo comprising gelatin capsules containing lactose. A total of 48 subjects (38 men and 10 women) completed the study, which was divided into seven sessions. In the first session, three men were given malathion at a dose of 0.5 mg/kg bw. In the second session, another three men were given malathion at a dose of 1.5 mg/kg bw. Subsequently, seven men were given malathion at a dose of 5.0 mg/kg bw. Three and four men received malathion at a dose of 10 mg/kg bw in two separate sessions. There were three sessions in which three males, four males and seven females all received malathion at a dose of 15 mg/kg bw. In each session, one or more subjects received a placebo, which contained lactose (see Table 2 for experimental design). The subjects were kept under close observation from before dosing until 72 h after dosing. Any symptoms or clinical signs were recorded and blood pressure, pulse rate, respiratory rate and body temperature were determined the day before dosing, immediately before dosing and 2, 4, 8 and 24 h after dosing. Twelve-lead electrocardiograms (ECGs) were carried out 30 min before dosing and 2, 4, 8 and 24 h after dosing, and single channel continuous ECG was performed from 30 min before dosing until 4 h after dosing. Blood was collected for measurement of haematological and clinical chemical parameters at screening before entry into the study, before dosing and 24 h after dosing. Urine

Table 2. Design of a study in humans treated with a single dose of malathion

Session	No. of subjects ^a	Dose (mg/kg bw)					
		0 (placebo)	0.5	1.5	5.0	10.0	15.0
1	4 men	1	3	—	—	—	—
2	4 men	1	—	3	—	—	—
3	10 men	3	—	—	7	—	—
4	4 men	1	—	—	—	3	—
5	9 men	2	—	—	—	4	3
6	7 men	3	—	—	—	—	4
7	10 women	3	—	—	—	—	7

From Gillies & Dickson (2000)

analysis was performed at screening and 24 h after dosing. Blood for measurement of plasma and erythrocyte cholinesterase activity was taken at screening and on days -9, -7, -5, -2 and -1 and at -30 min (before dosing). Samples were also taken at 1, 2, 4, 8, 12, 24 and 48 h after dosing and on days 4, 7 and 14 after dosing. After separation into erythrocytes and plasma, samples were stored at -70°C . A modified Ellman method was used to assay cholinesterase activity, and these results were compared with those for placebo controls. Blood samples for the estimation of concentrations of malathion and malaoxon were taken before dosing and 1, 2, 4, 8 and 12 h and 1, 2 and 3 days after dosing. Concentrations of malathion and malaoxon were measured in the plasma of subjects receiving the highest dose (the limit of quantification of the analytical method for both malathion and its oxon was approximately 100 ng/ml). Urine was collected over the following periods: from 12 h before dosing until dosing; for 12 h after dosing; from 12–24 h after dosing; and from 24–48 h after dosing. These samples were analysed for malathion monocarboxylic acid, malathion dicarboxylic acid, dimethyl phosphate, dimethyl thiophosphate and dimethyl dithiophosphate. Criteria for withdrawal from the study were: (i) for individual subjects, >25% inhibition of plasma or erythrocyte cholinesterase activity compared with baseline activity at two consecutive time-points; (ii) for cohorts, >15% inhibition of plasma or erythrocyte cholinesterase activity at two consecutive time-points.

No test-material-related clinical changes were seen and ECGs, haematology and clinical chemistry were unaffected by the malathion. No significant changes in plasma or erythrocyte cholinesterase activity were observed when compared with activities before dosing or activities measured for placebo controls at any dose. Plasma concentrations of malathion and malaoxon in subjects receiving the highest dose (15 mg/kg bw) were below the limit of quantification; for this reason, samples from subjects receiving lower doses of malathion were not analysed. As no test material-related effect was observed during the study, the NOAEL was considered to be 15 mg/kg bw. This study complied with GCP (CPMP/ICH/135/95) and GLP (OECD, United States EPA, 40 CFR 160) (Gillies & Dickson, 2000).

Comments

As a supplement to a study of developmental neurotoxicity, a study was undertaken on the effects of orally administered malathion on the activity of cholinesterase. In this study, single or repeated doses of malathion of up to 450 mg/kg bw were administered orally to pregnant rats, pre-weaning offspring at various stages of development, and young adults. The NOAEL for the study was 50 mg/kg bw. Inhibition of brain cholinesterase activity was observed in offspring of untreated females who were given a single direct dose of 150 mg/kg bw on postnatal day 11.

A study of developmental toxicity in rabbits was evaluated by the 1997 JMPR. This study was re-evaluated in 2002. Malathion was administered at a dose of 25, 50 or 100 mg/kg bw per day by gavage to groups of mated female rabbits during days 6–18 of gestation. The NOAEL for maternal toxicity was 25 mg/kg bw per day on the basis of decreased maternal body-weight gain during dosing. There was no difference in fertility, number of corpora lutea, implantation sites, litter size or fetal weight and length. The NOAEL was 100 mg/kg bw per day for fetal toxicity on the basis of the absence of developmental toxicity at any dose.

In a study of developmental neurotoxicity, malathion was administered orally to groups of mated female rats from day 6 of gestation to postnatal day 10 and to their offspring from postnatal day 11 to postnatal day 21 at doses of 5, 50 or 150 mg/kg bw per day. Behavioural assessments were performed on both dams and pups, in the latter at intervals up to postnatal day 60. The NOAEL for the study was 50 mg/kg bw per day for developmental neurotoxicity (slower surface righting reflex at the highest dose of 150 mg/kg bw per day on postnatal day 11, but not subsequently) and 150 mg/kg bw per day for maternal toxicity. It was considered that the effects observed were likely to have been caused by current treatment rather than any permanent developmental neurotoxic effect, because neurotoxicity was not observed in the offspring at later time-points in the study.

The results of tests for chromosomal aberrations in human lymphocytes and gene mutation in mouse lymphoma cells were positive at cytotoxic concentrations. A test for unscheduled DNA synthesis in vivo in male rats gave negative results. This is consistent with the conclusions of the 1997 JMPR, which recorded that although the results of some tests in vitro on malathion were positive, malathion was not genotoxic in vivo.

An acceptable¹ randomized double-blind placebo-controlled ascending single oral dose study was carried out in healthy men and women aged 18–50 years. Malathion was administered in gelatin capsules at a dose of 0.5, 1.5, 5, 10 or 15 mg/kg bw. No test-material-related clinical changes were seen, nor were ECGs, haematology or clinical chemistry parameters affected by treatment with malathion. No significant changes in plasma or erythrocyte cholinesterase activities were observed when compared with activities before dosing or placebo controls at any dose. As no test-material-related effects were observed during the study, the NOAEL was considered to be 15 mg/kg bw.

After considering the new data made available to the Meeting and also the previous monograph, the Meeting established an acute RfD of 2 mg/kg bw on the basis of the study in humans and a safety factor of 10. It should be noted that this acute RfD is likely to be conservative as erythrocyte cholinesterase was more sensitive to inhibition by malathion than brain cholinesterase in the studies available to the Meeting. The Meeting considered that the use of data from studies in which pre-weaning pups received bolus doses of pesticides by direct dosing, particularly when they were also receiving the pesticide in unknown amounts from the dams via their milk, was inappropriate for the establishment of an acute RfD.

Estimate of acute RfD

2 mg/kg bw

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

Further observations in humans

¹ Annex 1, references 83, page 5.

References

- Edwards, C.N. (2001a) Malathion technical *in vitro* mammalian chromosome aberration test performed with human lymphocytes. Unpublished report No. 40412 from Scantox, Lille Skensved, Denmark. Submitted to WHO by Cheminova Agro A/S, Lemvig, Denmark.
- Edwards, C.N. (2001b) Malathion technical *in vitro* mammalian cell gene mutation test performed with mouse lymphoma cells (L5178Y). Unpublished report No. 40413 from Scantox, Lille Skensved, Denmark. Supplied to WHO by Cheminova Agro A/S, Lemvig, Denmark.
- Environmental Protection Agency (1996) Standard operating procedure for measuring cholinesterase in laboratory rats and dogs exposed to non-reversible cholinesterase inhibitors. *Federal Register, April 26th*, **61**, 18593.
- Fulcher, S.M., Hazelden, K.P., Renaut, S., Collier, M.J., Clemson, A.D. & Smith, M.J. (2001) Malathion effects on cholinesterase in the CD rat (adult and juvenile) by oral gavage administration. Unpublished report No. CHV067/012452 from Huntingdon Life Sciences, Alconbury Weston, England. Submitted to WHO by Cheminova Agro A/S, Lemvig, Denmark.
- Fulcher, S.M., Bottomley, A.M., Renaut, S., Collier, M.J., Taylor, I. & Clemson, A.D. (2002) Malathion developmental neurotoxicity study in the CD rat by oral gavage administration. Unpublished report No. CHV066/013331, from Huntingdon Life Sciences, Alconbury Weston, England. Submitted to WHO by Cheminova Agro A/S, Lemvig, Denmark.
- Fulcher, S.M. (2003) Malathion effects on cholinesterase in the CD rat (adult and juvenile) by oral gavage administration. Amendment 1 to the final report. Unpublished report No. CHV067/012452 (amendment 1), dated 12th June 2003, from Huntingdon Life Sciences, Alconbury Weston, England. Submitted to WHO by Cheminova Agro A/S, Lemvig, Denmark.
- Gillies, D. & Dickson, J. (2000) A randomised double blind ascending single dose study with malathion to determine the no effect level on plasma and RBC cholinesterase activity. Unpublished report No. ICR 013177, from Inveresk Research, Tranent, Scotland. Submitted to WHO by Cheminova Agro A/S, Lemvig, Denmark.
- Meerts, I.A.T.M. & van de Waart, E.J. (2003) Evaluation of DNA repair inducing ability of Fyfanon technical in male rat hepatocytes (*in vivo* rat hepatocyte DNA-repair assay). Unpublished revised report No. 342776, from Notox BV, s'Hertogenbosch, the Netherlands. Submitted to WHO by Cheminova Agro A/S, Lemvig, Denmark.
- Robinson, K. (2002) Evaluation of the embryo-lethal potential of malathion in the rabbit. Unpublished report, ClinTrials BioResearch Ltd, Senneville, Quebec, Canada. Submitted to WHO by Cheminova Agro A/S, Lemvig, Denmark.
- Siglin, J.C. (1985) A teratology study with AC 6,601 in rabbits. Unpublished report number 8171, from Food and Drug Research Laboratories Inc., Waverly, New York, USA. Submitted to WHO by Cheminova, DK-7620, Lemvig, Denmark.