3.3 MALATHION (49)

TOXICOLOGY

Malathion is the ISO-approved common name for *S*-1,2-bis(ethoxycarbonyl)ethyl *O*,*O*-dimethyl phosphorothioate (IUPAC), with the CAS number 121-75-5.

Malathion is a non-systemic organophosphorus insecticide whose mode of pesticidal action is the inhibition of cholinesterase activity. It is used to control insects on agricultural crops and stored commodities and for vector control.

The toxicity of malathion was evaluated by JMPR in 1963, 1965, 1966, 1997 and 2003. Malathion was listed in the periodic review programme of CCPR but was not yet scheduled for review. The compound was reviewed by the present Meeting following the recommendation of an electronic task force of the WHO Core Assessment Group on Pesticide Residues that it be re-evaluated due to public health concerns identified by IARC and the availability of a significant number of new studies.

The current Meeting evaluated all previously submitted toxicological data in addition to new published and unpublished toxicological studies and published epidemiological studies on cancer outcomes. All critical unpublished studies contained certificates of compliance with GLP, unless otherwise specified. Human volunteer studies were conducted according to the Declaration of Helsinki or equivalent ethical standards.

Biochemical aspects

In a study conducted in rats using [^{14}C]malathion, gastrointestinal absorption was at least 77% in males and 86% in females. The majority (up to 90%) of radioactivity was excreted in urine within 24 hours. Less than 1% of radioactivity was detected in tissues, with the highest proportions in the liver, skin, fat and gastrointestinal tract. There was no evidence that malathion or its metabolites accumulated in any tissue.

Malathion is extensively metabolized via desulfuration, oxidation, hydrolysis, dealkylation and demethylation reactions. In particular, the oxidative desulfuration of malathion in the liver generates malaoxon, which is a more potent inhibitor of acetylcholinesterase compared with malathion. The major metabolites detected in rat urine (> 80% of urinary radioactivity) were α- and β-monocarboxylic acids (MMCA) and the dicarboxylic acid (MDCA) of malathion. Other urinary metabolites include desmethyl malathion, *O*,*O*-dimethyl phosphorothioic acid, fumaric acid, 2-mercaptosuccinic acid, *O*,*O*-dimethyl phosphorodithioic acid, monoethyl fumarate and malaoxon. Malaoxon was observed only in urine samples and accounted for less than 2% of total urinary radioactivity. Similar metabolites were detected in human studies.

Published in vitro studies have further investigated the metabolism of malathion. In human liver microsomes, the metabolism of malathion to malaoxon was catalysed by CYP1A2, CYP2B6 or CYP3A4, their respective contributions depending on the concentration of malathion. Isomalathion, a storage impurity, was a potent non-competitive inhibitor of hepatic carboxylesterase activity, important for the formation of MMCA by human liver microsomes.

Estimates of in vitro dermal absorption through human skin ranged from 1.44% to 8.74% and from 8% to 20.7%. In a volunteer study, dermal absorption was 4.48% following a single application and 3.53% following a second application.

Toxicological data

Consistent with other organophosphorus insecticides, the most sensitive toxicological effect following acute and repeated exposures to malathion is the inhibition of acetylcholinesterase activity in erythrocytes and brain. At higher doses, cholinergic signs become evident.

In rats, the oral LD_{50} ranged from 1539 to 8227 mg/kg bw, the dermal LD_{50} was greater than 2000 mg/kg bw and the inhalation LC_{50} was greater than 5.2 mg/L. The dermal LD_{50} in rabbits was 8790 mg/kg bw. Malathion was slightly irritating to rabbit skin and eyes. In a Buehler test conducted in guinea-pigs, malathion did not cause skin sensitization, whereas malathion caused skin sensitization in the guinea-pig maximization test. Malathion was not sensitizing in the mouse local lymph node assay.

In a 14-day range-finding study conducted in juvenile rats, which tested gavage malathion doses of 0, 250, 450 and 600 mg/kg bw per day, salivation occurred at 450 and 600 mg/kg bw per day. In males, erythrocyte and brain acetylcholinesterase activities were reduced at every dose, whereas in females, erythrocyte and brain acetylcholinesterase activities were reduced at 450 and 600 mg/kg bw per day.

In a 28-day repeated-dose toxicity study in rats, which tested dietary malathion concentrations of 0, 100, 500, 5000 and 10 000 ppm (equal to 0, 9.2, 46.1, 457.5 and 947.8 mg/kg bw per day for males and 0, 9.4, 47.4, 461.3 and 910.1 mg/kg bw per day for females, respectively), the NOAEL was 500 ppm (equal to 46.1 mg/kg bw per day) for the inhibition of erythrocyte and brain acetylcholinesterase activities at 5000 ppm (equal to 457.5 mg/kg bw per day). Nasal toxicity, consisting of goblet cell depletion and hyperplasia of the olfactory epithelium, was noted at the highest dose.

In a 30-day repeated-dose toxicity study in rats, which tested dietary malathion concentrations of 0, 50, 100, 500, 10 000 and 20 000 ppm (equal to 0, 5.1, 10.4, 51.9, 1036 and 2008 mg/kg bw per day for males and 0, 5.7, 11.6, 57.6, 1134 and 2193 mg/kg bw per day for females, respectively), the NOAEL was 500 ppm (equal to 51.9 mg/kg bw per day) for the inhibition of brain acetylcholinesterase activity at 10 000 ppm (equal to 1036 mg/kg bw per day).

The overall NOAEL from these two 1-month repeated-dose toxicity studies in rats was 500 ppm (equal to 51.9 mg/kg bw per day), with an overall LOAEL of 5000 ppm (equal to 457.5 mg/kg bw per day).

In a 90-day repeated-dose toxicity study in rats, which tested dietary malathion concentrations of 0, 100, 500, 5000, 10 000 and 20 000 ppm (equal to 0, 7, 34, 340, 680 and 1390 mg/kg bw per day for males and 0, 8, 39, 384, 784 and 1597 mg/kg bw per day for females, respectively), the NOAEL was 500 ppm (equal to 34 mg/kg bw per day) for the inhibition of brain acetylcholinesterase activity at 5000 ppm (equal to 340 mg/kg bw per day).

In a second 90-day repeated-dose toxicity study in rats, which tested dietary malathion concentrations of 0, 100, 500, 5000 and 10 000 ppm (equal to 0, 7.2, 35.0, 353.6 and 733.8 mg/kg bw per day for males and 0, 7.5, 35.9, 363.1 and 719.0 mg/kg bw per day for females, respectively), the NOAEL was 100 ppm (equal to 7.2 mg/kg bw per day) for goblet cell depletion at 500 ppm (equal to 35.0 mg/kg bw per day). This is considered to be an atypical result, as the effect is likely to have arisen through non-dietary exposure.

In a 13-week neurotoxicity study in rats, which tested dietary malathion concentrations of 0, 50, 5000 and 20 000 ppm (equal to 0, 4, 352 and 1486 mg/kg bw per day for males and 0, 4, 395 and 1575 mg/kg bw per day for females, respectively), the NOAEL was 50 ppm (equal to 4 mg/kg bw per day), based on the inhibition of erythrocyte acetylcholinesterase activity at 5000 ppm (equal to 352 mg/kg bw per day).

The overall NOAEL for the 90-day (neuro)toxicity studies in rats was 500 ppm (equal to 34 mg/kg bw per day) for effects at 5000 ppm (equal to 340 mg/kg bw per day).

In a 28-day range-finding study in dogs in which malathion was administered orally in capsules at doses of 0, 125, 250 and 500 mg/kg bw per day, inhibition of erythrocyte acetylcholinesterase occurred at 250 and 500 mg/kg bw per day, with deaths, cholinergic signs and reduced body weight and feed consumption occurring at the highest dose.

In a 12-month repeated-dose toxicity study in dogs in which malathion was administered orally in capsules at doses of 0, 62.5, 125 and 250 mg/kg bw per day, the NOAEL was 125 mg/kg bw per day for reduced body weight and haematological changes at 250 mg/kg bw per day. Inhibition of erythrocyte acetylcholinesterase activity occurred at every dose but was of marginal toxicological significance in the absence of brain acetylcholinesterase inhibition.

In a 3-week repeated-dose dermal toxicity study in rabbits, which tested malathion doses of 0, 50, 300 and 1000 mg/kg bw per day, the NOAEL was 300 mg/kg bw per day for the inhibition of brain acetylcholinesterase activity at 1000 mg/kg bw per day.

In a 21-day repeated-dose dermal toxicity study in rabbits, which tested malathion doses of 0, 75, 100, 150 and 500 mg/kg bw per day, the NOAEL was 150 mg/kg bw per day for the inhibition of brain acetylcholinesterase activity at 500 mg/kg bw per day.

In a 13-week repeated-dose inhalational toxicity study in which rats were exposed whole body to an aerosol malathion concentration of 0, 0.1, 0.45 or 2.0 mg/L, a no-observed-adverse-effect concentration (NOAEC) was not determined, as laryngeal hyperplasia and degeneration and/or hyperplasia of the olfactory epithelium occurred at every concentration.

In an 18-month pre-GLP study conducted in mice, which tested dietary malathion concentrations of 0, 8000 and 16 000 ppm (equivalent to 0, 1200 and 2400 mg/kg bw per day, respectively), a NOAEL for chronic toxicity was not identified, because clinical signs during the second year of exposure and reduced body weight occurred at both doses. Although no treatment-related tumours were observed, this study was considered unreliable for assessing carcinogenicity because of the small number of concurrent control mice (n = 10) compared with the treated groups (n = 50).

In a second 18-month study conducted in mice, which tested dietary malathion concentrations of 0, 100, 800, 8000 and 16 000 ppm (equal to 0, 17, 143, 1476 and 2978 mg/kg bw per day for males and 0, 21, 167, 1707 and 3448 mg/kg bw per day for females, respectively), the NOAEL for chronic toxicity was 800 ppm (equal to 143 mg/kg bw per day) for the inhibition of brain acetylcholinesterase activity at 8000 ppm (equal to 1476 mg/kg bw per day). Increases in liver carcinomas in males at the low dose and second highest dose were not considered treatment related because of the lack of a dose–response relationship, the lack of corroboration in females and the fact that liver carcinomas are a common age-related tumour in this strain of mouse (B6C3F1). The NOAEL for carcinogenicity was 800 ppm (equal to 143 mg/kg bw per day) for an increased incidence of liver adenomas at 8000 ppm (equal to 1476 mg/kg bw per day).

In an 80-week pre-GLP study conducted in rats, which tested dietary malathion concentrations of 0, 4700 and 8150 ppm (equivalent to 0, 1200 and 2400 mg/kg bw per day, respectively), it was not possible to identify a NOAEL for chronic toxicity because of the lack of reporting detail. While there was an increase in proliferative lesions of the thyroid in both sexes at both doses, these increases were not statistically significant in males and were significant in females only in a trend test and not by pairwise comparison when compared with groups of pooled controls. Overall, this study is not considered acceptable for the assessment of carcinogenicity because of the small number of rats in the concurrent control group (15 versus 50 in the treated groups) and the short duration of exposure.

In a subsequent 24-month pre-GLP study conducted in rats, which tested dietary malathion concentrations of 0, 100, 1000 and 5000 ppm (equivalent to 0, 5, 50 and 250 mg/kg bw per day, respectively, as calculated by a previous Meeting), the NOAEL was 100 ppm (equivalent to 5 mg/kg bw per day) for the inhibition of erythrocyte acetylcholinesterase activity at 1000 ppm (equivalent to 50 mg/kg bw per day). The NOAEL for carcinogenicity was 5000 ppm (equivalent to 250 mg/kg bw per day), the highest dose tested.

In a 24-month chronic toxicity and carcinogenicity study in rats, which tested dietary malathion concentrations of 0, 100, 500, 6000 and 12 000 ppm (equal to 0, 7, 29, 359 and 729 mg/kg bw per day for males and 0, 8, 35, 415 and 868 mg/kg bw per day for females, respectively), the NOAEL for

chronic toxicity was 500 ppm (equal to 29 mg/kg bw per day) for reduced red cell parameters, inhibition of brain acetylcholinesterase activity and the occurrence of nasal toxicity at 6000 ppm (equal to 359 mg/kg bw per day). The nasal toxicity was characterized by olfactory epithelial degeneration, hyperplasia and cyst formation, goblet cell hyperplasia, congestion, oedema and inflammation. Four nasal adenomas were observed, one in each sex at the two highest doses. In females, but not males, the incidence of liver adenomas was increased slightly at 6000 and 12 000 ppm, but the incidences were within the performing laboratory's historical control range. A NOAEL of 500 ppm (equal to 29 mg/kg bw per day) was identified for carcinogenicity, based on the increase in nasal adenomas at 6000 ppm (equal to 359 mg/kg bw per day).

The Meeting concluded that there is some evidence that malathion is carcinogenic in rats and mice.

The Meeting noted that the mouse liver adenomas observed in the second 18-month study occurred at doses exceeding the maximum tolerated dose and were not replicated in other mouse studies. The increases in liver adenomas in rats observed in the 24-month chronic toxicity and carcinogenicity study occurred only in females and were within the performing laboratory's historical control range. Whereas the rodent liver adenomas were co-incident with liver hypertrophy, there were no findings in these or other studies to suggest a possible mode of action, such as liver enzyme induction or cytotoxicity. Malathion showed no peroxisome proliferator–activated receptor alpha or gamma activity and also showed no aryl hydrocarbon receptor activity. Overall, the Meeting considered that there was equivocal evidence to suggest a tumorigenic response in the liver, but this had a clear threshold and was likely to be secondary to the effects on the liver of prolonged exposure to very high dietary concentrations of malathion.

Based on consistent observations of nasal toxicity in dietary studies of various durations ranging from 28 days to 2 years and in a short-term inhalational toxicity study, the Meeting concluded that the formation of nasal adenomas in rats was due to a local mechanism of irritancy and cytotoxicity caused by prolonged exposure of the nasal epithelium to high concentrations of malathion absorbed via inhaled food particles or as a vapour arising from food. This produces a state of reactive hyperplasia, a causative factor in tumour formation. Scenarios of prolonged, direct and excessive exposure of human nasal tissue to malathion or malathion metabolites following ingestion of residues is unlikely, and therefore these tumours would not occur in humans following exposure to malathion in the diet.

Malathion has been extensively tested for genotoxicity using a broad range of in vitro and in vivo assays. In 1997, the Meeting evaluated the available unpublished and published genotoxicity studies and noted that the majority of studies indicated that malathion is not genotoxic, although a small number of studies indicated that it can induce chromosomal aberrations and sister chromatid exchanges in vitro. However, there was no evidence that malathion induced chromosomal aberrations in vivo. Therefore, the 1997 Meeting concluded that malathion does not induce genotoxic damage in vivo. The 2003 Meeting evaluated supplementary genotoxicity studies and found that malathion caused chromosomal aberrations in cultured human lymphocytes and gene mutations in the mouse lymphoma assay at cytotoxic concentrations, but did not cause unscheduled DNA synthesis in vivo in male rats. The 2003 Meeting reaffirmed its previous conclusion that although the results of some in vitro tests were positive, malathion was not considered to induce genotoxic damage in vivo.

In addition to the studies considered at previous meetings, the current Meeting considered a number of new published and unpublished genotoxicity studies, including studies that involved the assessment of genotoxic damage in exposed workers. Many of the published studies do not provide adequate experimental detail, do not specify the purity of the malathion tested or were conducted on commercial formulations, or used in vivo test systems or exposure routes less relevant to the risk assessment of dietary residues of pesticides. The following discussion is limited to studies that evaluated technical malathion or malathion at purities above 90% and provided adequate experimental and data analysis details to allow interpretation of the findings.

Using standard genotoxicity test systems, malathion was not mutagenic in assays using prokaryotes or lower eukaryotes when tested with or without metabolic activation. In contrast, in in vitro assays using either human or non-human cells, malathion was generally positive for the induction of (1) chromosomal damage, as measured by increased frequencies of chromosomal aberrations or micronuclei; (2) mutations; and (3) DNA damage, as measured by increases in DNA migration in the alkaline comet assay and increased frequencies of sister chromatid exchanges. Negative findings were reported for the induction of micronuclei in Molt-4 T-lymphocytes, unscheduled DNA synthesis in WI-38 cells and primary rat liver hepatocytes, and mutations in a mouse lymphoma assay (reported to be equivocal without metabolic activation and negative with metabolic activation).

Using in vivo non-mammalian systems, malathion was active for micronucleus induction in a bird model and for induction of reciprocal translocations and sex-linked recessive lethals in one *Drosophila melanogaster* study, but not for sex-linked recessive lethals, sex chromosome loss or wing spot mutations in another study.

Based on the criteria mentioned in section 2.1, very few of the 34 in vivo mammalian study/end-point combinations were considered adequate for this review. In reports submitted by the sponsor, malathion was negative in a rat liver unscheduled DNA synthesis study when administered by gavage, in a rat bone marrow chromosomal aberration study when administered by gavage and in a mouse bone marrow erythrocyte micronucleus assay when administered intraperitoneally. However, the unscheduled DNA synthesis assay is insensitive for detecting genotoxic compounds; the micronucleus assay, as conducted, suffers from concerns about scoring criteria; and the chromosomal aberration test appears to be significantly underpowered, based on the frequency of chromosomal aberrations detected among control and treated animals. A negative mouse dominant lethal test was also reported when malathion was administered in feed for 7 weeks, and a negative mouse bone marrow chromosomal aberration study was reported in intraperitoneally treated mice. In contrast, malathion-induced micronuclei and chromosomal aberrations were reported in bone marrow immature erythrocytes and proliferating cells, respectively. A positive alkaline comet assay using blood leukocytes sampled from rats treated intraperitoneally once a day for 5 days was reported.

The Meeting evaluated a number of human studies that examined genotoxicity end-points. Patients treated for acute intoxication with a malathion-based product exhibited increased levels of chromosomal damage in lymphocytes. The frequency of micronuclei and glycophorin A mutations in erythrocytes or micronuclei in lymphocytes was not increased in workers exposed selectively to malathion. However, DNA damage and chromosomal aberrations have been reported in workers exposed to a mixture of pesticides, including malathion. These studies are of limited value for examining the specific effect of malathion on genotoxicity end-points in humans.

The Meeting noted that malathion has been reported to have genotoxic activity in multiple assay systems at multiple genetic end-points. In several studies where evaluated, reactive oxygen species appear to have been responsible for the increased damage, as demonstrated by the detection of malathion-induced 8-hydroxy-2′-deoxyguanosine and increased malondialdehyde concentrations in isolated human peripheral blood mononuclear cells treated in vitro, an effect attenuated by co-treatment with *N*-acetylcysteine or curcumin; by increased intracellular levels of reactive oxygen species and reduced levels of catalase, superoxide dismutase and glutathione in rat PC12 cells treated in vitro, an effect ameliorated by co-treatment with vitamin E; and by the detection of oxidative damage using the comet assay in isolated rat lymphocytes treated in vitro with malathion. Supportive of this hypothesis, malathion appears to selectively induce markers of oxidative stress in Tox21/ToxCast high-throughput screening assays. The Meeting concluded that the observed genotoxic effects occur secondary to the formation of reactive oxygen species, which will exhibit a threshold.

The Meeting concluded that malathion is unlikely to be genotoxic at anticipated dietary exposures.

In the multigeneration and developmental toxicity studies, cholinesterase activity was not measured.

In a two-generation reproductive toxicity study conducted in rats, which tested dietary malathion concentrations of 0, 550, 1700, 5000 and 7500 ppm (equal to 0, 43, 130, 393 and 595 mg/kg bw per day for males and 0, 50, 152, 438 and 655 mg/kg bw per day for females, respectively), the NOAEL for both reproductive toxicity and parental toxicity was 7500 ppm (equal to 595 mg/kg bw per day), the highest dose tested. The NOAEL for offspring toxicity was 1700 ppm (equal to 130 mg/kg bw per day) for reduced pup weights at 5000 ppm (equal to 393 mg/kg bw per day).

Two published studies reported potential testicular toxicity in rats exposed to malathion orally, but these studies had a number of methodological limitations that reduced their utility. Further, the reported observations are not corroborated by the preceding GLP-compliant multigenerational rat study in which no effects on the testes were observed.

A variety of in vivo and in vitro assays in mammalian and non-mammalian models indicated that malathion is unlikely to affect the endocrine system.

In a pilot developmental toxicity study in rats, which tested gavage malathion doses of 0, 300, 600, 800 and 1000 mg/kg bw per day from days 6 to 15 of gestation, no embryo or fetal toxicity occurred, whereas maternal toxicity occurred at and above 600 mg/kg bw per day. In the main developmental toxicity study in rats, which tested gavage doses of 0, 200, 400 and 800 mg/kg bw per day from days 6 to 15 of gestation, the NOAEL for maternal toxicity was 400 mg/kg bw per day for clinical signs and reduced body weight gain and feed consumption at 800 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 800 mg/kg bw per day, the highest dose tested.

In a range-finding developmental toxicity study in rabbits, which tested gavage malathion doses of 0, 25, 50, 100, 200 and 400 mg/kg bw per day from days 6 to 18 of gestation, no embryo or fetal toxicity occurred, whereas maternal toxicity occurred at 200 and 400 mg/kg bw per day. In the main study, which tested malathion doses of 0, 25, 50 and 100 mg/kg bw per day from days 6 to 18 of gestation, the NOAEL for maternal toxicity was 25 mg/kg bw per day for a marginal effect on body weight gain at 50 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 100 mg/kg bw per day, the highest dose tested.

The Meeting concluded that malathion is not teratogenic.

In a study conducted in hens, there was no evidence that malathion caused delayed peripheral neuropathy.

In an acute neurotoxicity study in rats, which tested gavage malathion doses of 0, 500, 1000 and 2000 mg/kg bw, the NOAEL was 1000 mg/kg bw for reduced erythrocyte acetylcholinesterase activity in females and reduced ambulatory activity in males at 2000 mg/kg bw.

A 13-week neurotoxicity study in rats is described above together with the other 13-week toxicity studies in rats, and an overall NOAEL is identified for these studies.

In a developmental neurotoxicity study in rats, which tested gavage malathion doses of 0, 5, 50 and 150 mg/kg bw per day from day 6 of gestation to day 10 of lactation, the NOAEL for both maternal toxicity and offspring toxicity was 50 mg/kg bw per day for clinical signs at 150 mg/kg bw per day.

Administration of malathion from day 6 of gestation to day 21 of lactation had no effect on the thickness of the corpus callosum in rat pups at doses up to 150 mg/kg bw per day.

The Meeting concluded that malathion is neurotoxic.

Studies in rats have examined the time to peak effect and compared the effects of malathion and malaoxon on the inhibition of acetylcholinesterase activity. The time to peak effect in juvenile rats following dosing with malathion ranged from 30 to 90 minutes for the inhibition of erythrocyte acetylcholinesterase activity and from 60 to 90 minutes for the inhibition of brain

acetylcholinesterase activity. Malaoxon was a more potent inhibitor of acetylcholinesterase activity compared with malathion. Comparison of benchmark doses (BMDs) following acute oral dosing indicated that the toxicity adjustment factor (TAF) for malaoxon was 21.5 in males and 17.4 in females for the inhibition of erythrocyte acetylcholinesterase activity and 14.8 in males and 11.0 in females for the inhibition of brain acetylcholinesterase activity. Comparison of BMDs for the inhibition of erythrocyte acetylcholinesterase activity from chronic toxicity studies indicated that TAFs for malaoxon ranged from 37 to 38 in males and from 65 to 69 in females.

In a 6-week immunotoxicity study in female rats, which tested dietary malathion concentrations of 0, 50, 100, 700 and 7000 ppm (equal to 0, 8.9, 17.6, 126.8 and 1215.8 mg/kg bw per day, respectively), the NOAEL for immunotoxicity was 7000 ppm (equal to 1215.8 mg/kg bw per day), the highest dose tested.

The Meeting concluded that malathion is not immunotoxic.

An extensive literature search did not identify any potential adverse effects on intestinal microbiota or any evidence that intestinal microbiota can metabolize malathion.

Toxicological data on metabolites, degradates and/or impurities

Current FAO specifications for malathion prescribe maximum limits for isomalathion (CAS No. 3344-12-5), malaoxon (CAS No. 152-20-05), *O*,*O*,*S*-trimethyl phosphorothioate (CAS No. 2953-29-9) and *O*,*S*,*S*-trimethyl phosphorodithioate (CAS No. 152-18-1).

Toxicity tests were conducted on malaoxon, isomalathion, desmethyl malathion, desmethyl malathion monocarboxylic acid, MMCA, MDCA and desmethyl malaoxon dicarboxylic acid.

Malaoxon

The oral LD_{50} in rats for malaoxon was 50 mg/kg bw.

In a 14-day range-finding study in rats, which tested malaoxon at dietary concentrations of 0, 10, 25, 100, 2500 and 3500 ppm (equal to 0, 1.1, 3.0, 12.1, 293 and 387 mg/kg bw per day for males and 0, 1.1, 3.1, 12.5, 281.6 and 294.7 mg/kg bw per day for females, respectively), inhibition of erythrocyte acetylcholinesterase activity occurred at and above 100 ppm (equal to 12.1 mg/kg bw per day). At the two highest doses, inhibition of brain acetylcholinesterase activity and reduced body weight gain and feed consumption occurred.

In a 103-week carcinogenicity study conducted in mice, which tested dietary malaoxon concentrations of 0, 500 and 1000 ppm (estimated by a previous Meeting to be equal to 0, 75 and 150 mg/kg bw per day, respectively), survival and body weight were reduced at the highest dose. There were no treatment-related neoplastic or non-neoplastic lesions. In a parallel study conducted in rats, which tested the same dietary concentrations of malathion (equal to 0, 25 and 50 mg/kg bw per day, respectively), the combined incidence of C-cell adenomas and carcinomas of the thyroid in females was increased, although this was comparable to historical control values. The incidence of gastric ulcers, commonly observed in the forestomach, was increased in treated rats.

In a 24-month toxicity study in rats, which tested malaoxon at dietary concentrations of 0, 20, 1000 and 2000 ppm (equal to 0, 1, 57 and 110 mg/kg bw per day for males and 0, 1, 68 and 140 mg/kg bw per day for females, respectively), the NOAEL for chronic toxicity was 20 ppm (equal to 1 mg/kg bw per day), based on mortality and the inhibition of brain acetylcholinesterase activity at 1000 ppm (equal to 57 mg/kg bw per day). The NOAEL for carcinogenicity was 2000 ppm (equal to 110 mg/kg bw per day), the highest dose tested. Similar to studies conducted on malathion, inflammatory changes in the nasal mucosa occurred at 1000 and 2000 ppm; these changes were likely attributable to inhaled food particles containing malaoxon, resulting in tissue injury and inflammation of the nasal cavity, with secondary effects on the lungs and middle ear.

The Meeting concluded that malaoxon is not carcinogenic in mice or rats.

Malaoxon was negative for mutagenicity in bacterial assays and in lower eukaryotes, both with and without metabolic activation. Malaoxon was reported to be active for induction of sister chromatid exchanges but not chromosomal aberrations in Chinese hamster ovary cells, with or without metabolic activation. An increase in sister chromatid exchanges when tested in the absence of metabolic activation only was also reported; it was also reported that malaoxon was more potent than malathion in this assay. Malaoxon was also reported to induce DNA damage as measured by the comet assay in rat adrenal gland PC12 cells when tested in the absence of metabolic activation only and was mutagenic in mouse lymphoma (L5178Y) cells in the absence but not the presence of metabolic activation. In this study, there seemed to be a preference for the induction of small colonies, generally considered to be indicative of chromosomal damage rather than gene mutations.

Malaoxon induced DNA damage in isolated lymphocytes in the absence of metabolic activation, as measured by the alkaline comet assay; studies with metabolic activation were not conducted. Further, a follow-up study concluded that the malaoxon-mediated damage was likely induced by reactive oxygen species. Also, malaoxon is more potent than malathion in inducing intracellular levels of reactive oxygen species and reducing levels of catalase, superoxide dismutase and glutathione in rat PC12 cells treated in vitro, an effect ameliorated by co-treatment with vitamin E. Also, similar to malathion, malaoxon appears to selectively induce markers of oxidative stress in Tox21/ToxCast high-throughput screening assays. When provided in food, malaoxon induced an increase in reciprocal translocations and sex-linked recessive lethals in *D. melanogaster*, but not for sex-linked recessive lethals when administered by injection. Malaoxon was reported negative for the induction of chromosomal aberrations and sister chromatid exchanges in the bone marrow cells of male mice following a single intraperitoneal injection.

The Meeting concluded that the observed genotoxic effects occur secondary to the formation of reactive oxygen species, which will exhibit a threshold.

The Meeting concluded that malaoxon is unlikely to be genotoxic at anticipated dietary exposures.

Other metabolites

The oral LD_{50} in rats was greater than 2000 mg/kg bw for desmethyl malathion, desmethyl malathion monocarboxylic acid, MMCA, MDCA and desmethyl malaoxon dicarboxylic acid. The oral LD_{50} in rats for desmethyl malaoxon dicarboxylic acid, trisodium salt, was greater than 2000 mg/kg bw.

There are a limited number of genotoxicity studies on other metabolites of malathion. MDCA, MMCA, desmethyl malathion monocarboxylic acid, potassium salt, and desmethyl malaoxon dicarboxylic acid, trisodium salt, as well as isomalathion, *O*,*O*,*O*-trimethyl phosphorothioate, *O*,*O*,*S*-trimethyl phosphorothioate and *O*,*S*,*S*-trimethyl phosphorodithioate, were reported negative for bacterial mutagenicity, with and without metabolic activation. Isomalathion induced DNA damage in isolated lymphocytes in the absence of metabolic activation, as measured by the alkaline comet assay; studies with metabolic activation were not conducted. Isomalathion was also reported to induce micronuclei in the human liver–derived HepaRG cell line.

Using quantitative structure–activity relationships, the storage impurity, 2-mercaptosuccinic acid diethyl ester, was determined to have no greater toxicity than malathion.

The potential of malathion metabolites to inhibit acetylcholinesterase activity has been studied in rats. Comparisons of erythrocyte acetylcholinesterase activities indicated that desmethyl malathion, MMCA and MDCA are at least 2.75-, 1.9- and 4.6-fold less potent than malathion.

Based on a comparison of the inhibitions of acetylcholinesterase activities over acute and chronic exposure durations and a comparison of BMDs (see above), the Meeting concluded that malaoxon is approximately 30-fold more potent than malathion.

Human data

As in laboratory animals, the inhibition of acetylcholinesterase activity is the most sensitive adverse effect in humans exposed to malathion, mediated through the metabolite malaoxon, which is a more potent inhibitor of acetylcholinesterase activity compared with malathion. A comparative in vitro study indicated that malaoxon was a slightly less potent inhibitor (< 2-fold) of human compared with rat acetylcholinesterase activity.

In a study conducted in male and female volunteers, which tested single oral doses of malathion at 0, 0.5, 1.5, 5, 10 and 15 mg/kg bw, the NOAEL was 15 mg/kg bw, the highest dose tested, based on the absence of any adverse effects, including the inhibition of erythrocyte acetylcholinesterase activity. In a subsequent study conducted in male and female volunteers, which tested single oral doses of malathion of 0, 0.5, 1.5, 5.0, 10.0 and 15.0 mg/kg bw, there were no treatment-related adverse events or effects on erythrocyte acetylcholinesterase activity.

In a published study, application of malathion to the forearm of human volunteers increased blood flow, mediated via the inhibition of acetylcholinesterase activity.

In a published non-blinded study, slight inhibition of erythrocyte acetylcholinesterase activity occurred in children following two applications of a 1% malathion shampoo used to treat head lice.

In a 1994 summary report, there were no poisoning incidents and no inhibition of plasma cholinesterase activity in workers involved in the manufacture of malathion over a 20-year period. In a subsequent (1999) summary report, biological monitoring of workers employed at dimethoate and malathion manufacturing plants from 1994 to 1999 detected no reduction in plasma cholinesterase activity.

Several epidemiological studies on cancer outcomes in relation to occupational exposure to malathion were available. The evaluation of these studies focused on the occurrence of NHL and prostate cancer, as outlined in section 2.2. One meta-analysis was available, as well as one prospective cohort study, the AHS, with a large sample size and detailed exposure assessment. Cohort studies are considered a powerful design, as recall bias is avoided. All other studies were case–control studies, usually retrospective, which are more prone to recall and selection biases.

The AHS found no evidence of a positive association of NHL with malathion exposure or of an exposure–response relationship. In contrast, various case–control studies reported excess risks of NHL associated with use of malathion. In a large pooled case–control study, the unadjusted estimates showed a significant increased risk of NHL (RR = 1.6; 95% CI = 1.2–2.2) associated with ever versus never use of malathion. However, these were attenuated and/or no longer significant when proxy respondents were excluded and analyses were mutually adjusted for other pesticides. Significant elevated risks of NHL were reported from the cross-Canada case–control study of pesticides and health for ever versus never use of malathion (OR = 1.96; 95% CI = 1.42–2.70) and when examining annual days of use, although there was no clear exposure–response relationship across exposure categories. Non-significant increased risks of NHL were reported by two other case–control studies, one of which had limited statistical power based on only five exposed cases. The meta-analysis, which did not include the AHS, found a significant 80% excess risk ratio for ever versus never use of malathion.

Overall, there is some very weak evidence of a positive association between malathion exposure and NHL from the case–control studies and the overall meta-analysis. However, it is notable that the AHS, which is the only cohort study and is large and of high quality, found no evidence of an association at any exposure level.

There was no evidence of an association with all prostate cancers and malathion exposure in the AHS. However, a significant excess risk of aggressive prostate cancer (RR = 1.43; 95% CI = 1.08–1.88) in the highest exposure category (highest quintile of intensity-weighted lifetime days of malathion exposure), along with a significant exposure–response relationship (P for trend = 0.04), was observed. A significant elevated risk of all prostate cancer was observed in a case–control study

for ever use (OR = 1.34; 95% CI = 1.01–1.78) and for highest lifetime cumulative exposure versus those unexposed (OR = 1.49; 95% CI = 1.02–2.18). A significant trend across exposure categories ($P = 0.03$) was also reported. However, interpretation of results from this study is limited by potential for exposure misclassification in the job–exposure matrix used for exposure assessment and by the potential for residual confounding from lack of adjustment for other pesticide exposures. There was no evidence of an association between prostate cancer and malathion exposure in the United Farm Workers of America study, which was limited by the use of ecological rather than individual-level exposure assessment.

Overall, the evidence is suggestive of a positive association between malathion exposure and risk of aggressive prostate cancer; however, the evidence base is limited to the one large AHS cohort study.

Based on a consideration of the results of animal bioassays, genotoxicity assays and epidemiological data from occupational exposures, the Meeting concluded that malathion and its metabolites are unlikely to pose a carcinogenic risk to humans from exposure via the diet.

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

Toxicological evaluation

The current Meeting reaffirmed the ADI of 0–0.3 mg/kg bw, based on the NOAEL of 500 ppm (equal to 29 mg/kg bw per day) in the 2-year study of toxicity and carcinogenicity in rats for the inhibition of brain acetylcholinesterase and using a 100-fold safety factor, established by the 1997 Meeting. The margins of exposure between this ADI and the doses causing liver adenomas in mice and nasal adenomas in rats are 5000-fold and 1200-fold, respectively.

The current Meeting reaffirmed the ARfD of 2 mg/kg bw, based on the NOAEL of 15 mg/kg bw for the inhibition of erythrocyte acetylcholinesterase activity in a study conducted in male and female volunteers with the application of a 10-fold safety factor, established by the 2003 Meeting. This ARfD is supported by the NOAEL of 15 mg/kg bw in a second study conducted in male and female volunteers. The ARfD is considered to be a conservative value, because human acetylcholinesterase is slightly less sensitive (< 2-fold) than rat acetylcholinesterase to malaoxon.

The Meeting concluded that the metabolite malaoxon is approximately 30-fold more toxic than malathion. On this basis, a 30-fold potency factor should be applied to the residue levels for use in both the acute and chronic dietary exposure estimates for malaoxon, and these should be added to the dietary exposures for malathion and compared with the ARfD and ADI for malathion, respectively.

Both the ADI and ARfD are established for the sum of malathion and malaoxon (corrected for its potency), expressed as parent malathion. The other metabolites of malathion considered by the present Meeting are less potent than the parent compound and therefore would be covered by the ADI and ARfD for malathion. The impurity isomalathion may need to be taken into consideration in the risk assessment depending on its concentration in food commodities.

A toxicological monograph was prepared.

Levels relevant to risk assessment of malathion

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year study of toxicity and carcinogenicity[a]	Toxicity	800 ppm, equal to 143 mg/kg bw per day	8 000 ppm, equal to 1 476 mg/kg bw per day

Species	Study	Effect	NOAEL	LOAEL
		Carcinogenicity	800 ppm, equal to 143 mg/kg bw per day	8 000 ppm, equal to 1 476 mg/kg bw per day
Rat	Acute neurotoxicity study[b]	Toxicity	1 000 mg/kg bw per day	2 000 mg/kg bw per day
	One-month studies of toxicity[a,c]	Toxicity	500 ppm, equal to 51.9 mg/kg bw per day	5 000 ppm, equal to 457.5 mg/kg bw per day
	Thirteen-week studies of toxicity and neurotoxicity[a,c]	Toxicity	500 ppm, equal to 34 mg/kg bw per day	5 000 ppm, equal to 340 mg/kg bw per day
	Two-year study of toxicity and carcinogenicity[a]	Toxicity	500 ppm, equal to 29 mg/kg bw per day	6 000 ppm, equal to 359 mg/kg bw per day
		Carcinogenicity	500 ppm, equal to 29 mg/kg bw per day	6 000 ppm, equal to 359 mg/kg bw per day
	Two-generation study of reproductive toxicity[a,e]	Reproductive toxicity	7 500 ppm, equal to 595 mg/kg bw per day[d]	–
		Parental toxicity	7 500 ppm, equal to 595 mg/kg bw per day[d]	–
		Offspring toxicity	1 700 ppm, equal to 130 mg/kg bw per day	5 000 ppm, equal to 393 mg/kg bw per day
	Developmental toxicity study[b,e]	Maternal toxicity	400 mg/kg bw per day	800 mg/kg bw per day
		Embryo and fetal toxicity	800 mg/kg bw per day[d]	–
	Developmental neurotoxicity study[b,e]	Maternal toxicity	50 mg/kg bw per day	150 mg/kg bw per day
		Offspring toxicity	50 mg/kg bw per day	150 mg/kg bw per day
Rabbit	Developmental toxicity study[b,e]	Maternal toxicity	25 mg/kg bw per day	50 mg/kg bw per day
		Embryo and fetal toxicity	100 mg/kg bw per day[d]	–
Dog	One-year study of toxicity[f]	Toxicity	125 mg/kg bw per day	250 mg/kg bw per day
Human	Acute volunteer studies[c,f]	Cholinesterase inhibition	15 mg/kg bw[d]	–

[a] Dietary administration.
[b] Gavage administration.
[c] Two or more studies combined.
[d] Highest dose tested.
[e] Acetylcholinesterase activity not measured.
[f] Capsule administration.

Estimate of acceptable daily intake (ADI)

0–0.3 mg/kg bw (for sum of malathion and malaoxon, adjusted for its potency, and expressed as malathion)

Estimate of acute reference dose (ARfD)

2 mg/kg bw (for sum of malathion and malaoxon, adjusted for its potency, and expressed as malathion)

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

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

Results from in vivo genotoxicity studies investigating oral dosing, because malathion genotoxicity data are highly variable and inconsistent and there is a lack of robust in vivo rodent studies using the oral route of exposure

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

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid; > 77%
Dermal absorption	Estimates vary (1.44–20.7% in human skin)
Distribution	Rapid tissue distribution
Potential for accumulation	No potential for accumulation
Rate and extent of excretion	Rapid and complete
Metabolism in animals	Extensive; oxidation, hydrolysis, dealkylation and demethylation reactions
Toxicologically significant compounds in animals and plants	Malathion, malaoxon, desmethyl malathion, desmethyl malaoxon, MMCA, MDCA, isomalathion
Acute toxicity	
Rat, LD_{50}, oral	> 1 539 to < 8 227 mg/kg bw
Rat, LD_{50}, dermal	> 2 000 mg/kg bw
Rat, LC_{50}, inhalation	> 5.2 mg/L
Rabbit, dermal irritation	Slightly irritating
Rabbit, ocular irritation	Slightly irritating
Guinea-pig, dermal sensitization	Not sensitizing (Buehler assay)
	Sensitizing (maximization assay)
Mouse, dermal sensitization	Not sensitizing (local lymph node assay)
Short-term studies of toxicity	
Target/critical effect	Acetylcholinesterase inhibition
Lowest relevant oral NOAEL	51.9 mg/kg bw per day (28 days; rat)
Lowest relevant dermal NOAEL	150 mg/kg bw per day (21 days; rabbit)
Lowest relevant inhalation NOAEC	< 0.1 mg/L (13 weeks; rat)
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Acetylcholinesterase inhibition

Lowest relevant NOAEL	29 mg/kg bw per day (rat)
Carcinogenicity	Some evidence of carcinogenicity in mice and rats[a]
Genotoxicity	
	Genotoxic, possibly due to the generation of reactive oxygen species[a]
Reproductive toxicity	
Reproduction target/critical effect	No effect on reproduction
Lowest relevant parental NOAEL	595 mg/kg bw per day (rat; highest dose tested)[b]
Lowest relevant offspring NOAEL	130 mg/kg bw per day (rat)[b]
Lowest relevant reproduction NOAEL	595 mg/kg bw per day (rat; highest dose tested)[b]
Developmental toxicity	
Developmental target/critical effect	Marginally reduced maternal body weight gain
Lowest maternal NOAEL	25 mg/kg bw per day (rabbit)[b]
Lowest embryo/fetal NOAEL	100 mg/kg bw per day (rabbit; highest dose tested)[b]
Neurotoxicity	
Acute neurotoxicity NOAEL	1 000 mg/kg bw
Subchronic neurotoxicity NOAEL	4 mg/kg bw per day[c]
Developmental neurotoxicity NOAEL	50 mg/kg bw per day[b]
Delayed neurotoxicity	No evidence
Other toxicological studies	
Immunotoxicity NOAEL	1 216 mg/kg bw per day (rat; highest dose tested)
	Not immunotoxic
Toxicological studies on malaoxon	
Rat, LD_{50}, oral	50 mg/kg bw
Lowest relevant long-term NOAEL	1 mg/kg bw per day (rat)
Carcinogenicity	No evidence of carcinogenicity (mouse, rat)
Genotoxicity	Some evidence of genotoxicity, secondary to the formation of reactive oxygen species
Toxicological studies on desmethyl malathion, sodium salt	
Rat, LD_{50}, oral	> 2 000 mg/kg bw
Genotoxicity	Not mutagenic in prokaryotic assays
Toxicological studies on desmethyl malathion monocarboxylic acid, potassium salt	
Rat, LD_{50}, oral	> 2 000 mg/kg bw
Genotoxicity	Not mutagenic in prokaryotic assays
Toxicological studies on MMCA	
Rat, LD_{50}, oral	> 2 000 mg/kg bw
Genotoxicity	Not mutagenic in prokaryotic assays

Toxicological studies on MDCA	
Rat, LD_{50}, oral	> 2 000 mg/kg bw
Genotoxicity	Not mutagenic in prokaryotic assays
Toxicological studies on desmethyl malaoxon dicarboxylic acid	
Rat, LD_{50}, oral	> 2 000 mg/kg bw
Genotoxicity	Not mutagenic in prokaryotic assays
Human data	Acetylcholinesterase inhibition:
	Acute NOAEL: 15 mg/kg bw, highest dose tested
	No adverse effects in manufacturing personnel

[a] Unlikely to pose a carcinogenic risk to humans via exposure from the diet.
[b] Acetylcholinesterase activity not measured.
[c] Ninety-day neurotoxicity study in rats is covered by the overall oral NOAEL for repeated-dose studies of toxicity.

Summary

	Value	Studies	Safety factor
ADI	0–0.3 mg/kg bw	Two-year chronic toxicity and carcinogenicity study (rat)	100
ARfD	2 mg/kg bw	Single-dose studies (humans)	10

DIETARY RISK ASSESSMENT

The current residue definition for the estimation of dietary exposure is malathion. The Meeting identified that malaoxon is approximately 30 times more potent than malathion based on the end-point (acetylcholinesterase inhibition) on which the ADI and ARfD have been established. Malaoxon is generally present in food at concentrations that are approximately 3% of the malathion concentration. If malaoxon were included in the residue definition for dietary risk assessment, the exposures calculated below for comparison with the health-based guidance values would be approximately double.

Long-term dietary exposure

The ADI for malathion is 0–0.3 mg/kg bw. The IEDIs for malathion were estimated for the 17 GEMS/Food cluster diets using the STMR or STMR-P values estimated by JMPR. The results are shown in Annex 3. The IEDI ranged from 0.1% to 0.5% of the maximum ADI. The Meeting concluded that the long-term dietary exposure to residues of malathion from uses that have been considered by JMPR is unlikely to present a public health concern.

Short-term dietary exposure

The ARfD for malathion is 2 mg/kg bw. The IESTI for malathion was calculated for the plant commodities for which STMR and HR levels were estimated by the 1999, 2004 and 2008 JMPRs and for which consumption data were available. The results are shown in Annex 4. The calculated IESTIs were 0–5% of the ARfD for the general population and 0–9% of the ARfD for children. The Meeting concluded that the short-term dietary exposure to malathion residues from uses considered by the Meeting was unlikely to present a public health concern.

6. RECOMMENDATIONS

The Meeting recommended that a guidance document be developed for the evaluation of genotoxicity studies, taking the experience gained from this meeting into account.