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### 5.3 AZINPHOS METHYL (002)

#### TOXICOLOGY

Azinphos-methyl is the ISO approved common name for *S*-3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-ylmethyl *O,O*-dimethyl phosphorodithioate (IUPAC) or *O,O*-dimethyl *S*-[(4-oxo-1,2,3-benzotriazin-3(4*H*)-yl)methyl] phosphorodithioate (CAS; CAS No. 86-50-0). It is a broad-spectrum organophosphorus pesticide. Its toxicity was first evaluated by the 1965 JMPR, when an ADI of 0–0.0025 mg/kg bw was established based on a NOAEL of 0.25 mg/kg bw per day for inhibition of cholinesterase activity in serum and erythrocytes in a repeat-dose study in rats. The 1968 JMPR considered a number of additional studies that were not available to the Meeting in 1965. The ADI established in 1965 was confirmed. The 1973 JMPR considered new studies that involved human volunteers but, owing to the absence of sufficient information on the conduct of these studies in humans, the existing ADI was re-affirmed. In 1991, the ADI was increased to 0–0.005 mg/kg bw on the basis of reduced body-weight gain and fertility observed in the multigeneration study in rats. Azinphos-methyl was reviewed by the present Meeting within the Periodic Re-evaluation Programme of the CCPR. All studies previously submitted to JMPR were available for consideration by the present Meeting. Several new studies, including two double-blind clinical studies in human volunteers, were also considered by the present Meeting.

Most studies, excluding some described in previous JMPR monographs, were certified as having been performed in compliance with good laboratory practice (GLP) and in accordance with the relevant Organization for Economic Co-operation and Development (OECD) test guidelines. The studies in humans were conducted in accordance with the principles of good clinical practice and the Declaration of Helsinki, or equivalent statements prepared for use by national and/or multinational authorities.

### ***Biochemical aspects***

After oral administration of radiolabelled azinphos-methyl, the radiolabel was rapidly and completely (90–100% of the administered dose) absorbed from the mammalian intestinal tract, widely distributed throughout the organs, and eliminated in the urine (60–70% of the administered dose) and faeces via bile (25–35% of the administered dose) within 48 h. The maximum concentration in blood was reached within 2–3 h after administration. Azinphos-methyl was rapidly cleared from the blood and tissues, and consequently there is negligible potential for accumulation. In rats, the initial steps of metabolism involved the formation of the highly reactive oxon metabolite and mercaptomethylbenzazimide by cytochrome P450. Glutathionyl methylbenzazimide and desmethyl isoazinphos-methyl were formed via glutathione transferase. Subsequent hydrolysis of glutathionyl methylbenzazimide resulted in the formation of cysteinyl-methylbenzazimide, which was then oxidized to form its corresponding sulfoxide and sulfone.

### ***Toxicological data***

Azinphos-methyl was highly acutely toxic (LD<sub>50</sub> range, 4.4–26 mg/kg bw) when administered orally in an aqueous or non-aqueous vehicle to rats, and its profile of clinical signs was similar to those of other cholinesterase-inhibiting organophosphorus pesticides. Clinical signs observed in experimental animals after acute exposure were salivation, lacrimation, vomiting, diarrhoea, anorexia, reduced locomotor activity, piloerection, staggering gait and muscular tremors. These signs were generally evident within 5–20 min after dosing. There was very little difference in the sensitivity of male and female rats to the acute effects of azinphos-methyl.

The main toxicological findings in repeat-dose studies in rodents and dogs were inhibition of cholinesterase activity and, at higher doses, reduced body-weight gain and signs of neurotoxicity. In short-term studies of toxicity of less than 12 months duration, the NOAEL for inhibition of erythrocyte acetylcholinesterase activity was 0.2 mg/kg bw per day in rats and dogs. The NOAEL for inhibition of brain cholinesterase activity was 0.9 mg/kg bw per day in rats, and 0.7 mg/kg bw per day in dogs. Toxicity observed in rats and dogs was limited to the characteristic muscarinic signs (diarrhoea, salivation) and reduced body-weight gain. The effect doses for these clinical signs in short-term studies correlated with the high levels of inhibition of brain cholinesterase activity (> 80%) in rats and dogs.

Azinphos-methyl was tested in an adequate range of studies of genotoxicity *in vitro* and *in vivo* and showed no evidence of genotoxicity. The Meeting concluded that azinphos-methyl is unlikely to be genotoxic.

In long-term studies of toxicity, inhibition of cholinesterase activity was again the main toxicological finding in mice and rats. In mice, erythrocyte and brain cholinesterase activities were inhibited at 3.8 mg/kg bw per day, with a NOAEL of 0.9 mg/kg bw per day. Reduced body-weight gain and clinical signs involving hyperactivity and convulsions were observed in mice at higher doses (6.25 mg/kg bw per day). At equivalent doses in rats, body tremors and deaths were reported, although reduced body-weight gain was observed at 2.7 mg/kg bw per day. In rats, the NOAEL for inhibition of erythrocyte acetylcholinesterase activity was 0.3 mg/kg bw per day, while for brain cholinesterase activity it was 0.9 mg/kg bw per day and the NOAEL for a reduction in body-weight gain was 0.9 mg/kg bw per day. There was no evidence of carcinogenicity with azinphos-methyl at dietary concentrations of up to 40 ppm (equal to 12.8 mg/kg bw per day) in mice and up to 45 ppm (equal to 2.7 mg/kg bw per day) in rats; these were the highest doses tested.

In the absence of any carcinogenic potential in rodents and the lack of genotoxic potential *in vitro* and *in vivo*, the Meeting concluded that azinphos-methyl is unlikely to pose a carcinogenic risk to humans.

In multigeneration studies of reproductive toxicity in rats, the treatment-related effects of azinphos-methyl were cholinergic signs at high doses, reductions in body-weight gain and inhibition

of cholinesterase activity. These effects were consistent with those seen in short- and long-term studies of toxicity. However, there was also evidence of reduced pup viability at 4.8 mg/kg bw per day. The NOAEL for inhibition of brain cholinesterase activity in dams was 5 ppm, equal to 0.5 mg/kg bw per day. The NOAEL for inhibition of brain cholinesterase activity in pups was 15 ppm, equal to 1.5 mg/kg bw per day.

In studies of developmental toxicity with azinphos-methyl in mice, rats and rabbits, teratogenicity was not observed at doses of up to 2, 3.6 and 15 mg/kg bw per day respectively. The only developmental effect noted in any of these studies was delayed ossification in rat and rabbit fetuses at doses that also caused inhibition of brain and erythrocyte cholinesterase activity in the dams. The NOAEL for developmental effects in fetuses was 2 mg/kg bw per day in rats and 1.5 mg/kg bw per day in rabbits. Inhibition of brain cholinesterase activity was not observed in rats at doses of 1 mg/kg bw per day or in rabbits at doses of 2.5 mg/kg bw per day.

In studies of delayed neurotoxicity, azinphos-methyl was administered to chickens either as a single dose at up to 330 mg/kg bw or as repeated doses of up to 225 mg/kg bw per day in the feed for 30 days; there was no evidence of delayed neuropathy.

In rats given azinphos-methyl as a single dose at up to 12 mg/kg bw by gavage or as repeated doses of up to 7.4 mg/kg bw per day in the diet for 13 weeks, cholinergic signs and significant inhibition of erythrocyte and brain cholinesterase activity were seen at a number of doses. In these studies, which included a functional observational battery (FOB) of tests, clinical signs of intoxication (perianal stain, red lacrimation, increased reactivity, uncoordinated gait, tremor) were observed. However, cholinergic signs were observed only when brain cholinesterase activity was inhibited by more than 70% or when erythrocyte acetylcholinesterase activity was inhibited by approximately 65–80%. This occurred after repeated doses in excess of 3.2 mg/kg bw per day or after a single dose of 6 mg/kg bw. The NOAEL for inhibition of cholinesterase activity in the brain after a single dose was 2 mg/kg bw or 1 mg/kg bw per day after repeat dosing.

In a randomized double-blind study in human volunteers (seven of each sex) given ascending single oral doses, azinphos-methyl did not induce cholinergic signs or changes in acetylcholinesterase activity in erythrocytes at doses of up to 1 mg/kg bw in males and up to 0.75 mg/kg bw in females; these were the highest doses tested.

When eight male volunteers were given azinphos-methyl as a daily oral dose at 0.25 mg/kg bw per day for 28 days, there were no cholinergic signs and erythrocyte acetylcholinesterase activity was not significantly inhibited. In another study, two groups of five male volunteers were given azinphos-methyl at a dose of 0.26 or 0.29 mg/kg bw per day for 30 days did not induce cholinergic signs or changes in cholinesterase activity in erythrocytes or plasma. In a third study, a similar outcome was reported when two male volunteers were given azinphos-methyl orally at a dose of 0.23 mg/kg bw per day for 30 days.

Regular medical examinations of workers involved in formulating products containing azinphos-methyl had revealed no effects, except for one case of possible dermatosis resulting in sensitive dry skin.

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

### **Toxicological evaluation**

The Meeting established an ADI of 0–0.03 mg/kg bw per day based on a NOAEL of 0.29 mg/kg bw per day for the absence of inhibition of erythrocyte acetylcholinesterase activity in a 30-day study of toxicity in male volunteers and a safety factor of 10. Since the database indicated that rodents and dogs of each sex and humans had similar NOAEL values for the most sensitive end-point, namely inhibition of acetylcholinesterase activity in erythrocytes, the NOAELs identified in the studies in humans were considered to be protective for the entire population. The Meeting also considered the

ADI to be protective for other, non-neurotoxic effects of azinphos-methyl observed in short- and long-term studies with repeated doses, and in studies of reproductive and developmental toxicity, where the use of a safety factor of 100 would be appropriate. The effect of azinphos-methyl on body-weight gain and fertility in dams at 15 ppm (0.5 mg/kg bw per day) in multigeneration studies of reproduction in rats was reconsidered. The Meeting concluded that it was a marginal effect that could not be directly attributed to treatment.

The Meeting established an ARfD of 0.1 mg/kg bw based on the NOAEL of 1 mg/kg bw and using a safety factor of 10. The NOAEL observed in a study of single doses in volunteers was the highest tested dose in males. Although the maximum dose given to females was only 0.75 mg/kg bw, there was no apparent observed difference in sensitivity between the sexes, so the NOAEL observed in males was also considered to be protective of effects in females. In a study of acute neurotoxicity in rats, the NOAEL was 2 mg/kg bw on the basis of inhibition of cholinesterase activity in the brain. At a dose of 2 mg/kg bw, significant inhibition of acetylcholinesterase activity in erythrocytes of male rats was observed, but not at 1 mg/kg bw in female rats. In rats, pup deaths as a result of inhibition of cholinesterase activity were observed at 6 mg/kg bw in females and at 12 mg/kg bw in males, suggesting a steep dose–response effect. Based on the median oral LD<sub>50</sub> value of 13 mg/kg bw (range, 4.4–26 mg/kg bw) in all available studies in rats, there would be about a 130-fold margin between the ARfD and the LD<sub>50</sub> in rats.

A toxicological monograph was prepared.

#### *Levels relevant for risk assessment*

Species	Study	Effect	NOAEL	LOAEL
Rat	Acute neurotoxicity <sup>a</sup>	Inhibition of brain cholinesterase activity	2.0 mg/kg bw	3.0 mg/kg bw
Human	Single dose <sup>b,d</sup>	No adverse effects	1.0 mg/kg bw per day	—
Mouse	Two-year study of toxicity and carcinogenicity <sup>c</sup>	Inhibition of erythrocyte and brain cholinesterase activity	5 ppm, equal to 0.9 mg/kg bw per day	20 ppm, equal to 3.8 mg/kg bw per day
		Carcinogenicity <sup>d</sup>	40 ppm, equal to 12.8 mg/kg bw per day	—
Rat	Three-month study of toxicity <sup>c</sup>	Inhibition of brain cholinesterase activity	0.9 mg/kg bw per day	3.4 mg/kg bw per day
	Two-year study of toxicity and carcinogenicity <sup>c</sup>	Reduced body-weight gain and inhibition of brain cholinesterase activity	15 ppm, equal to 0.9 mg/kg bw per day	45 ppm, equal to 2.7 mg/kg bw per day
		Carcinogenicity <sup>d</sup>	45 ppm, equal to 2.7 mg/kg bw per day	—
	Multigeneration study of reproductive toxicity <sup>e,e</sup>	Parental toxicity	5 ppm, equal to 0.5 mg/kg bw per day	15 ppm, equal to 1.5 mg/kg bw per day
		Offspring toxicity	15 ppm, equal to 1.5 mg/kg bw per day	45 ppm equal to 4.8 mg/kg bw per day
Developmental toxicity <sup>a,e</sup>	Maternal toxicity	1.0 mg/kg bw per day	2.0 mg/kg bw per day	

Species	Study	Effect	NOAEL	LOAEL
		Embryo/fetotoxicity	2.0 mg/kg bw per day	3.6 mg/kg bw per day
	Three-month study of neurotoxicity <sup>c</sup>	Inhibition of brain cholinesterase activity	15 ppm, equal to 1 mg/kg bw per day	45 ppm, equal to 3 mg/kg bw per day
Rabbit	Developmental toxicity <sup>b,c</sup>	Maternal toxicity	2.5 mg/kg bw per day	6.0 mg/kg bw per day
		Embryo/fetotoxicity	1.5 mg/kg bw per day	4.75 mg/kg bw per day
Dog	One-year study of toxicity <sup>a</sup>	Reduced body-weight gain and inhibition of brain cholinesterase activity	25 ppm, equal to 0.7 mg/kg bw per day	125 ppm, equal to 4.1 mg/kg bw per day
Human	Clinical 30-day study <sup>b,d,e</sup>	No adverse effects	0.29 mg/kg bw per day	—

<sup>a</sup> Gavage administration.

<sup>b</sup> Capsule administration.

<sup>c</sup> Dietary administration.

<sup>d</sup> Highest tested dose.

<sup>e</sup> Two or more studies combined

#### *Estimate of acceptable daily intake for humans*

0–0.03 mg/kg bw

#### *Estimate of acute reference dose*

0.1 mg/kg bw

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

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

#### ***Critical end-points for setting guidance values of exposure for azinphos-methyl***

##### *Absorption, distribution, excretion and metabolism in mammals*

Rate and extent of oral absorption	Almost complete absorption. Maximum plasma concentration 2–3 h after dosing.
Dermal absorption	See previous azinphos-methyl monographs
Distribution	Extensive
Potential for accumulation	Low, no evidence of accumulation
Rate and extent of excretion	Largely complete within 48 h; approximately 95% excreted in urine and bile.
Metabolism in animals	Extensive; two major urinary metabolites and six other products. Five faecal metabolites (10–12% of the administered dose).
Toxicologically significant compounds in	Azinphos methyl, azinphos-methyl oxon

animals, plants and the environment

*Acute toxicity*

Rat, LD <sub>50</sub> , oral	4.4–26 mg/kg bw
Rat, LD <sub>50</sub> , dermal	See previous azinphos-methyl monographs
Rat, LC <sub>50</sub> , inhalation	See previous azinphos-methyl monographs
Skin sensitization (test method used)	See previous azinphos-methyl monographs

*Short-term studies of toxicity*

Target/critical effect	Inhibition of brain cholinesterase activity
Lowest relevant oral NOAEL	0.7 mg/kg bw per day (dog)
Lowest relevant dermal NOAEL	See previous azinphos-methyl monographs
Lowest relevant inhalation NOAEC	See previous azinphos-methyl monographs

*Genotoxicity*

Unlikely to pose a genotoxic risk in vivo

*Long-term studies of toxicity and carcinogenicity*

Target/critical effect	Inhibition of brain cholinesterase activity
Lowest relevant NOAEL	0.9 mg/kg bw per day (rat)
Carcinogenicity	Not carcinogenic in rats and mice

*Reproductive toxicity*

Reproduction target/critical effect	Inhibition of brain cholinesterase activity in dams
Lowest relevant reproductive NOAEL	0.5 mg/kg bw per day (rats)
Developmental target/critical effect	Delayed ossification at maternally toxic doses (rats, rabbits)
Lowest relevant developmental NOAEL	1.0 mg/kg bw per day (rats); 2.5 mg/kg bw per day (rabbits)

Neurotoxicity/delayed neurotoxicity	No evidence of delayed neuropathy observed in hens NOAEL: 1 mg/kg bw in a repeat-dose study of neurotoxicity in rats
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*Medical data*

Medical examinations of workers involved in formulating azinphos-methyl products revealed no effects, except for one case of possible dermatosis resulting in sensitive dry skin.

*Summary*

	Value	Study	Safety factor
ADI	0–0.03 mg/kg bw per day	Humans, 30-day oral-dosing study	10
ARfD	0.1 mg/kg bw	Humans, study of acute toxicity	10