

#### 4. EVALUATION OF DATA FOR ACCEPTABLE DAILY INTAKE AND ACUTE DIETARY INTAKE FOR HUMANS, MAXIMUM RESIDUE LEVELS AND SUPERVISED TRIAL MEDIAN RESIDUE VALUES

##### 4.1 ACEPHATE (095)

#### TOXICOLOGY

Acephate is the International Organization for Standardization (ISO) approved name for the organophosphorus insecticide *O,S*-dimethyl acetylphosphoramidothioate, which is a cholinesterase inhibitor. The toxicology of acephate was evaluated by the Joint Meeting in 1976, 1982, 1984, 1987, 1988, 1990 and 2002. The 2002 JMPR established an ADI of 0–0.01 mg/kg bw based on the NOAEL of 10 ppm (equal to 0.58 mg/kg bw per day) in a 13-week study in rats and a safety factor of 50. The 2002 JMPR also established an ARfD of 0.05 mg/kg bw based on the NOAEL of 2.5 mg/kg bw in female rats in a study of acute neurotoxicity. The NOAELs were identified on the basis of inhibition of brain acetylcholinesterase activity. The overall safety factor of 50 ( $100/4 \times 2$ ) was applied, this being a combination of:

- a fourfold reduction in the safety factor because of the absence of relevant sex or species (including humans) differences in inhibition of cholinesterase activity or in kinetics, and the fact that the effect was dependent on the  $C_{\max}$ ;
- an additional safety factor of 2 for the marginal but statistically significant inhibition of brain cholinesterase activity observed in rats and dogs at 5 and 10 ppm.

The present Meeting re-evaluated acephate because new data had been submitted, including a study of metabolism in rats, a short-term study of neurotoxicity in rats, a study of developmental neurotoxicity in rats and a 28-day study in humans. The Meeting also reviewed relevant data from the previous evaluations.

All the new studies submitted for consideration by the Meeting complied with good laboratory practice (GLP).

#### ***Biochemical aspects***

In a new toxicokinetic study in rats given doses of 25 or 100 mg/kg bw by oral administration, acephate was rapidly absorbed with a time to maximum concentration in plasma ( $T_{\max}$ ) of 0.5–1 h. The terminal half-life was 1.4 h. There was no evidence of any persistent accumulation in tissue. Excretion in the urine accounted for 83–89% of the administered dose, most of this appearing in the first 6–12 h after dosing. Elimination via the faeces and as carbon dioxide accounted for about 2% and 5–9% of the administered dose, respectively. Most of the compound excreted in the urine during the first 24 h after dosing was unmetabolized acephate and  $\leq 5\%$  was methamidophos. Small amounts of *O*-desmethyl acephate, *O*-desmethyl methamidophos and *O,S*-dimethyl phosphorothioate, have also been identified in the urine.

The pharmacokinetics of acephate was similar in men and women given a single oral dose of 0.35–1.2 mg/kg bw. The  $T_{\max}$  for plasma was 1–4 h and the terminal elimination half-life was between 3.5 h and 6.6 h. Most of the recovered acephate and methamidophos was found in urine during the first 12 h after dosing. Methamidophos accounted for about 1.3% of the amount recovered in the urine, independently of the dose administered.

A comparison of dose administered and  $C_{max}$  in rats and humans is reported in Table 12. There were no relevant differences between humans and rats, considering the different methods used.

**Table 12: Relationship between dose administered and  $C_{max}$  in rats and humans**

	Humans				Rats	
Dose (mg/kg bw)	0.35	0.7	1	1.2	25	100
$C_{max}$ ( $\mu$ g/mL)	0.45	0.8	1.35	1.6	23	90

### Toxicological data

Acephate was a slightly more effective inhibitor of brain and erythrocyte acetylcholinesterase activities in rats ( $IC_{50}$  = 1.6 and 1.3 mmol/L, respectively) than in cynomolgus monkeys (concentration required to inhibit activity by 50%,  $IC_{50}$  = 3.4 and 2.7 mmol/L, respectively) or humans ( $IC_{50}$  = 5.4 and 2.7 mmol/L, respectively).

The median lethal dose ( $LD_{50}$ ) values were 1000–1400 mg/kg bw after oral administration in rats and > 10 000 mg/kg bw after dermal administration in rabbits. The  $LC_{50}$  value was > 15 mg/L air (4 h, nose-only) in rats. The clinical signs of toxicity corresponded to those typical of cholinergic poisoning.

In the new short-term study of neurotoxicity in rats fed diets containing acephate at a concentration of 50 to 1000 ppm, brain acetylcholinesterase activity was inhibited at the lowest dietary concentration tested (50 ppm), while erythrocyte acetylcholinesterase activity was reduced at dietary concentrations of 100 ppm and above. This confirms previous observations that in rats in vivo brain acetylcholinesterase is more sensitive to inhibition by acephate than is erythrocyte acetylcholinesterase. No clinical or neurobehavioural effects were observed at any dietary concentration, even the highest tested (1000 ppm), at which brain acetylcholinesterase activity was inhibited by about 80%. No NOAEL could be identified in this study, the LOAEL being 50 ppm (equal to 3.4 mg/kg bw per day).

This difference in enzyme sensitivity was not observed in dogs and monkeys. In a 52-week study, dogs were given diets containing acephate at concentrations of up to 800 ppm. There were no treatment-related clinical signs, no alterations in body weight or food consumption, no changes in ophthalmic parameters and no findings at gross necropsy. Brain and erythrocyte acetylcholinesterase activities were similarly inhibited, as shown in Table 13.

**Table 13. Mean percentage inhibition of acetylcholinesterase activity in dogs given diets containing acephate for 52 weeks**

Dietary concentration (ppm)	Brain acetylcholinesterase activity		Erythrocyte acetylcholinesterase activity	
	Males	Females	Males	Females
10	17	11	0	0
120	53	49	43	46
800	68	66	86	84

Similarly, the 1984 Meeting reported that in monkeys receiving acephate at a dose of 2.5 mg/kg bw per day by gavage for 33–34 days, the mean inhibition (relative to mean pre-treatment values) of acetylcholinesterase activity was 50% in erythrocytes and 47% in brain.

In the new study of developmental neurotoxicity, acephate was administered via gavage to pregnant rats from day 6 of gestation to postnatal day 6, and to pups from postnatal days 7 to 21. No significant inhibition of brain, erythrocyte or plasma cholinesterase activity was found in pups at postnatal day 4. At postnatal day 21, a significant reduction in brain acetylcholinesterase activity was observed at all doses. The degree of inhibition was found to be lower in erythrocytes and was significant at the highest dose only. A NOAEL could not be identified in this study.

Groups of seven volunteers received acephate as single oral doses at up to 1.2 mg/kg bw (men) and 1 mg/kg bw (women). No inhibition of erythrocyte acetylcholinesterase activity was reported in either sex at any dose. No clinically significant changes were seen in vital signs or on electrocardiography, haematology, clinical chemistry, urine analysis or physical examination. The NOAEL was 1.2 mg/kg bw per day, the highest dose tested.

In the new study in human volunteers, which was conducted according to current ethical standards, 10 men received acephate (purity, 99%) as daily oral doses at 0.25 mg/kg bw per day for 28 consecutive days. There was no inhibition of plasma cholinesterase or erythrocyte acetylcholinesterase activities at any time during the study. There were no treatment-related changes from baseline values for any haematology, clinical chemistry, electrocardiogram or urine analysis parameters, and no changes in vital signs or physical examination. The NOAEL was 0.25 mg/kg bw, the only dose tested.

### ***Toxicological evaluation***

To establish the ADI and ARfD, the Meeting considered the following elements derived from the available information:

The critical toxicological effect of acephate is the inhibition of acetylcholinesterase activity in the nervous system, an effect that is dependent on  $C_{max}$  rather than on the area under the curve (AUC).

Data on inhibition in vitro indicate that human brain acetylcholinesterase is slightly less sensitive to inhibition by acephate than is rat brain acetylcholinesterase.

Well conducted toxicokinetics studies, available for both rats and humans, show that there is no significant difference between the two species; in particular,  $C_{max}$  values have the same relationship to administered dose in the two species, and acephate is rapidly absorbed and eliminated in both species.

Data for rats in vivo indicate that inhibition of brain acetylcholinesterase activity occurs at lower doses than those required for a similar level of inhibition of erythrocyte acetylcholinesterase activity.

Data for dogs and monkeys in vivo indicate that brain and erythrocyte acetylcholinesterase activities are nearly equally inhibited at any given dose, and do not show the difference seen in rats, which might thus be rat-specific.

Well-conducted single- and repeated-dose studies in humans clearly show a NOAEL for inhibition of erythrocyte acetylcholinesterase activity.

Data from animals in vivo do not show sex differences in inhibition of acetylcholinesterase activity or clinical signs.

Studies in which acephate was administered by gavage (such as the study of developmental neurotoxicity in rats), while giving useful information, are not appropriate for setting an ADI because

repeated gavage administration to pups is not relevant to human long-term dietary exposure to residues of acephate.

The Meeting established an ADI of 0–0.03 mg/kg bw based on the NOAEL of 0.25 mg/kg bw per day from the study of repeated doses in humans and an overall safety factor of 10.

The Meeting established an ARfD of 0.1 mg/kg bw on the basis of the NOAEL of 1.2 mg/kg bw from the study of single doses in humans and an overall safety factor of 10.

The overall safety factor of 10 was derived by dividing the default value of 10 by 2 (because inhibition of acetylcholinesterase activity depends on the  $C_{max}$ ) and by multiplying by 2 (because some uncertainty remains with respect to the in-vivo sensitivity to inhibition of human brain acetylcholinesterase activity relative to that of erythrocyte acetylcholinesterase activity, since brain acetylcholinesterase may be more sensitive than erythrocyte acetylcholinesterase).

An addendum to the toxicological monograph was prepared.

#### *Levels relevant to risk assessment*

Species	Study	Effect	NOAEL	LOAEL
Rat	Acute neurotoxicity <sup>a,b</sup>	Toxicity	2.5 mg/kg bw	5 mg/kg bw
	Short-term study of neurotoxicity <sup>c</sup>	Toxicity	50 ppm, equivalent to 3.4 mg/kg bw per day	100 ppm, equivalent to 6.7 mg/kg bw per day
Rabbit	Developmental toxicity <sup>a</sup>	Maternal toxicity	3 mg/kg bw per day	10 mg/kg bw per day
		Embryo- and fetotoxicity	3 mg/kg bw per day	10 mg/kg bw per day
Dog	52-week study of toxicity <sup>c</sup>	Toxicity	10 ppm, equal to 0.27 mg/kg bw per day <sup>d</sup>	120 ppm, equal to 3.1 mg/kg bw per day
Human	Single-dose study <sup>e</sup>	Toxicity	1.2 mg/kg bw <sup>f</sup>	—
	28-day study <sup>e</sup>	Toxicity	0.25 mg/kg bw per day <sup>f</sup>	—

<sup>a</sup> Gavage administration

<sup>b</sup> Tested only in females

<sup>c</sup> Dietary administration

<sup>d</sup> Marginal effects on brain acetylcholinesterase activity, of equivocal toxicological relevance

<sup>e</sup> Oral administration

<sup>f</sup> Highest dose tested

#### *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 continued evaluation of the compound*

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

***Critical end-points relevant for setting guidance values for exposure to acephate***

<i>Absorption, distribution, excretion and metabolism in mammals</i>			
Rate and extent of oral absorption		Extensive and rapid	
Distribution		Widely distributed	
Potential for accumulation		None	
Rate and extent of excretion		Rapid and nearly completely, mainly via urine	
Metabolism in animals		Limited	
Toxicologically significant compounds (animals, plants and environment)		Acephate and methamidophos	
<i>Acute toxicity</i>			
Rat LD <sub>50</sub> oral		1000 mg/kg bw	
Rabbit LD <sub>50</sub> dermal		> 2000 mg/kg bw	
Rat LC <sub>50</sub> inhalation		> 15 mg/L air (4 h, nose-only)	
Skin irritation		Not irritating	
Eye irritation		Not irritating	
Skin sensitization (test method used)		Not sensitizing (Magnusson & Kligman)	
<i>Short-term studies of toxicity</i>			
Target/critical effect		Nervous system/inhibition of cholinesterase activity	
Lowest relevant oral NOAELa		10 ppm, equal to 0.58 mg/kg bw per day (13-week study in rats)	
<i>Genotoxicity</i>			
		Unlikely to be genotoxic in vivo	
<i>Long-term studies of toxicity and carcinogenicity</i>			
Target/critical effect		Nervous system/inhibition of cholinesterase activity	
Lowest relevant NOAEL		5 ppm, equivalent to 0.25 mg/kg bw per day (28-month study in rats)	
Carcinogenicity		Not likely to pose a carcinogenic risk to humans	
<i>Reproductive toxicity</i>			
Reproduction target/critical effect		Number of pups and postnatal survival decreased at parentally toxic doses	
Lowest relevant reproductive NOAEL		50 ppm (equivalent to 3.3 mg/kg bw per day)	
Developmental target/critical effect		Decreased fetal body weight and reduced ossification (rats) and slight developmental effects (rabbits) at maternally toxic doses; not teratogenic	
Lowest relevant developmental NOAEL		3 mg/kg bw per day (rabbits)	
<i>Neurotoxicity/delayed neurotoxicity</i>			
NOAEL for acute neurotoxicity		1.2 mg/kg bw (humans)	
NOAEL in short-term study of neurotoxicity		0.25 mg/kg bw per day (humans)	
		No signs of delayed polyneuropathy (hens)	
<i>Other toxicological studies</i>			
		Toxicokinetic and metabolism data not significantly different from data in rats	
<b><i>Summary</i></b>			
	Value	Study	Safety factor
ADI	0–0.03 mg/kg bw	Human, 28-day study	10
ARfD	0.1 mg/kg bw	Human, single-dose study	10

## DIETARY RISK ASSESSMENT

The current Meeting has established an ADI of 0–0.03 mg/kg bw and an ARfD of 0.1 mg/kg bw for acephate. In considering how to best approach the dietary risk assessment of mixed residues of acephate and methamidophos the 2003 JMPR decided that an appropriately conservative approach would be to sum the acephate and methamidophos residues after first scaling the methamidophos residues by a factor to account for the difference in toxicity. The current Meeting utilized the same approach, with relevant factors, for long and short-term intake, derived from the ratios of the acephate and methamidophos ADI and ARfD values; the factors are 7.5 and 10 respectively. Dietary intake estimates for the combined adjusted residues utilizing the new scaling factors were compared with the revised acephate ADI and ARfD.

### *Long-term intake*

The International Estimated Daily Intakes for the 5 GEMS/Food regional diets, based on estimated STMRS were in the range 1–7% of the maximum ADI of 0.03 mg/kg bw (Annex 3). The Meeting concluded that the long-term intake of residues of acephate from uses that have been considered by the 2003 JMPR is unlikely to present a public health concern.

### *Short-term intake*

The IESTI varied from 0% to 170% of the ARfD (0.1 mg/kg bw) for the general population and from 0% to 390% of the ARfD for children aged 6 years and below. The short-term intakes from apple, cauliflower and peppers were 110–170% of the ARfD for the general population and the short-term intakes from apple, broccoli, cauliflower, mandarin, nectarine, pear, peach and peppers were 130–390% of the ARfD for children aged 6 years and below. The information provided to the 2003 JMPR and re-evaluated in the current Meeting precluded a conclusion that the acute dietary intake of pome fruit (e.g. apple, pear) flowerhead brassicas (e.g. broccoli and cauliflower), mandarin, nectarine, peach and peppers would be below the ARfD.

The Meeting concluded that the short-term intake of residues of acephate from uses considered by the 2003 JMPR is unlikely to present a public health concern, with the exception of pome fruit (e.g. apple, pear) flowerhead brassicas (e.g. broccoli, cauliflower), mandarin, nectarine, peach and peppers.

## 4.2 AZOCYCLOTIN (067) AND CYHEXATIN (129)

### TOXICOLOGY

Azocyclotin (tri(cyclohexyl)-1H-1,2,4-triazole-1-yltin) and cyhexatin (tricyclohexyltin hydroxide) are chemically-related organotin compounds that are used as agricultural acaricides. Azocyclotin breaks down to cyhexatin and 1,2,4-triazole. Azocyclotin has similar systemic toxicological properties to cyhexatin and may also have additional properties attributable to the 1,2,4-triazole that is formed.

Toxicological data on cyhexatin were reviewed by the JMPR in 1970, 1973, 1977, 1978, 1980, 1981, 1988, 1989, 1991 and 1994. Azocyclotin was evaluated by the JMPR in 1974, 1981, 1989 and 1991. The Meeting in 1991 considered that the ADI for cyhexatin should also cover exposure to azocyclotin. In 1994, an ADI of 0–0.007 mg/kg bw was established based on a NOAEL of 0.7 mg/kg bw per day for reduced pup survival and decreased pup body-weight gain during lactation in a multigeneration study in rats.