ACEPHATE (addendum)

First draft prepared by
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Explanation

Acephate is the International Organization for Standardization (ISO) approved name for the organophosphorus insecticide *O,S*-dimethyl acetylphosphoramidothioate, which is an inhibitor of cholinesterase. The toxicology of acephate was evaluated by the Joint Meeting in 1976, 1982, 1984, 1987, 1988, 1990 and 2002. The 2002 JMPR established an acceptable daily intake (ADI) of 0–0.01 mg/kg bw based on the no-observed-adverse-effect level (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 acute reference dose (ARfD) of 0.05 mg/kg bw based on the NOAEL of 2.5 mg/kg bw in a study of acute neurotoxicity in female rats. The NOAELs were identified on the basis of inhibition of brain acetylcholinesterase activity. The overall safety factor of 50 (100 / 4 × 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).

Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Oral absorption, distribution, excretion and metabolism

In a new study of toxicokinetics that was conducted in compliance with the principles of GLP, groups of male and female Sprague-Dawley Crl:CD®(SD)IGS BR rats were given [^{14}C-*S*-methyl]acephate (radiochemical purity, > 95%) orally by gavage in deionized water. The study comprised two phases—a toxicokinetic phase and a metabolism and distribution phase.

In the toxicokinetic phase of the study, groups of three males and three females were given radiolabelled acephate as a single dose at 25 or 100 mg/kg bw, administered orally by gavage. The concentration of radioactivity in the plasma was measured between 0.5 h and 168 h after dosing.

At a dose of 25 mg/kg bw, the C_{max} in plasma was 22–25 μg of acephate equivalents per g plasma, the time to maximum concentration in plasma (T_{max}) was 0.5 h after dosing and the area under the curve of concentration–time (AUC_{0-168h}) was about 150 μg × h g^{-1} in both sexes. The toxicokinetics in animals receiving acephate at a dose of 100 mg/kg bw were proportional to those observed in animals receiving acephate at a dose of 25 mg/kg bw, the C_{max} in rat plasma being 83–98 μg/g acephate equivalents, T_{max} occurring at 0.5 h after dosing, and AUC_{0-168h} being 560 μg × h g^{-1}, respectively.

The rate of elimination from the plasma was multiphasic at each dose for both sexes, being characterized by a rapid elimination phase during the first 2 h after dosing, with the half-life for the initial phase of elimination estimated to be 1.4 h for male and female rats, and a terminal phase starting at about 24 h with a half-life of approximately 50 h.

In the metabolism and distribution phase of the study, groups of four male and four female rats were given radiolabelled acephate as an oral dose at 25 or 100 mg/kg bw and placed in glass metabolism cages. For each dose, separate groups were sacrificed at 0.5, 1, 2, 8, or 24 h after administration. Sampling times for the metabolism and distribution phase of the study were determined from results obtained in the toxicokinetic phase of the study. Concentrations of radioactivity were determined for blood and tissues of all animals, and for urine, faeces and exhaled air.

[^{14}C]Acephate was widely distributed in the tissues of both sexes after dosing at 25 or 100 mg/kg bw. The highest concentrations of radiolabel were observed 0.5–1.0 h after dosing, and were found in highly perfused organs, such as the liver, kidney and heart, which contained concentrations of radiolabel similar to those found in the plasma. Tissue concentrations decreased by an order of magnitude or more by 24 h after dosing. Actual concentrations of acephate equivalents attained during the 24 h after dosing ranged from 0.26 μg/g (in the fat of females at the lower dose at 24 h) to 108 μg/g (in the kidney of males at the higher dose at 0.5 h). Tissue concentrations attained were proportional to dose and independent of sex. The highest tissue concentration, expressed as a percentage of the administered dose, was about 3% in the liver of both sexes at 0.5 h after dosing. Most of the other tissues never contained more than 1% of the administered dose at any time after dosing, although higher concentrations were associated with non-absorbed material in the gastrointestinal tract. [^{14}C]Acephate equivalents in the brain of males and females never exceeded that of the blood cell fraction during the first 24 h after dosing. Concentrations of acephate in the blood cell fraction were about threefold higher than those in the brain at 0.5 h after dosing, and were roughly twofold higher at 24 h after dosing.

The pattern of elimination of acephate equivalents over the first 24 h after dosing was similar at both doses and in both sexes. Excretion in the urine accounted for 83–89% of the administered dose, while elimination via the faeces and as radiolabelled carbon dioxide was about 2% and 5–9%, respectively. Total recoveries of radioactivity were 98–101% and 92–93% for the group receiving the lowest dose and the group receiving the highest dose, respectively

Metabolism of acephate was minimal. Almost 90% of the radioactivity in the urine collected in the 24 h after dosing was unmetabolized acephate, regardless of dose or sex. In the group receiving acephate at a dose of 25 mg/kg, 9% of radioactivity was released as $^{14}CO_2$. Almost 90% of the radioactivity in the urine collected in the 24 h after dosing was unmetabolized acephate, regardless of dose or sex. Methamidophos accounted for about 5% of the radioactivity in the urine; however, since the material administered contained almost the same percentage of methamidophos, the metabolic origin is uncertain. Small amounts of other potential acephate metabolites *O, S*-dimethyl phosphorothioate, *O*-desmethyl acephate (*S*-methyl acetylphosphoramidothioate) and *O*-desmethyl methamidophos (*S*-methyl phosphoramidothioate) were also identified although, as with methamidophos, these compounds may have been impurities in the original dosing solution (Johnson, 2004).

After oral administration at a dose of 25 mg/kg bw per day for 7 days to rats, [*S*-methyl-^{14}C]acephate was rapidly absorbed and uniformly distributed. The highest concentrations of radiolabelled residues were found in the liver and skin. Most of the radioactive material recovered was excreted within 12 h. Urine contained 82–95% of the administered dose, 1–4% was exhaled, and 1% was found in the faeces. Less than 1% was found as a residue in tissues and organs 72 h after the last dose. Unchanged acephate (73–77%), *O*,*S*-dimethyl phosphorothioate (3–6%) and *S*-methyl acetylphosphoramidothioate (3–4%) were identified in the urine, but no methamidophos was found (Lee, 1972, summarized from 2002 JMPR).

2. Toxicological studies

2.1 Acute toxicity

The results of studies of the acute toxicity of acephate administered orally, dermally or by inhalation are summarized in Table 1 (this information was drawn from the evaluation made by the JMPR in 2002). The methods used in these studies complied to a certain extent with OECD guidelines. GLP was not compulsory when most of the studies were performed.

Table 1. Acute toxicity of acephate

Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	LC_{50} (mg/1 air)	Purity (%)
Rat	Sprague-Dawley	Males and females	Oral	1400 1000	—	97
Dog	Beagle	Males and females	Oral	210 [a] 680 [b]	—	89.6
Mouse	Not specified	Males and females	Oral	360	—	88.9
Rabbit	New Zealand	Male	Percutaneous	> 10 000	—	98.3
Rabbit	New Zealand	Males and females	Percutaneous	> 2000	—	97.8
Rat	Sprague-Dawley	Males and females	Inhalation (4 h)	—	> 15	97.8

Adapted from 2002 JMPR (Annex 1, reference 97)

[a] Minimum emetic dose

[b] Minimum lethal dose

The median lethal dose (LD_{50}) of acephate administered orally ranged from 360 mg/kg bw in mice to 1400 mg/kg bw in rats. The signs of poisoning were typical of the cholinergic syndrome. These included hypoactivity, tremors, muscular weakness and ruffed fur in mice and lethargy, excess salivation, lachrymation, exophthalmia, urinary incontinence, tremors, ataxia, diarrhoea, depression, collapse, bloody tears and/or decreased food consumption in rats. The signs of toxicity had disappeared by day 9 in the surviving mice and by day 8 in rats. No gross pathological alterations were seen in mice or rats at necropsy. The minimum emetic dose for beagle dogs was 220 mg/kg bw, and the minimum lethal dose was 680 mg/kg bw. All treated dogs showed excessive muscular tremors and diarrhoea. Animals at all doses above the lowest dose of 150 mg/kg bw had emesis and also dyspnoea, ataxia, clonic convulsions and bloody diarrhoea up to 6 h after dosing.

The LD_{50} for acephate after dermal administration in rabbits was > 10 000 mg/kg bw. All rabbits at this dose showed tremors shortly after dosing, and five had diarrhoea the following day.

The median lethal concentration (LC_{50}) value for rats treated by inhalation for 4 h with an aerosol of acephate dissolved in distilled water was > 15 mg/l. In Wistar rats, exposure of the nose and head to acephate at a mean concentration of 6.7 mg/l caused excess salivation and tremors in all animals on day 1, two rats showed ataxia, and two rats were lethargic on days 2 and 3. No other signs were recorded from day 4 onwards.

2.2 Short-term studies of toxicity

Groups of five male and five female beagle dogs received diets containing acephate (purity, 99.9%) at a concentration of 0, 10, 120 or 800 ppm (equal to 0, 0.27, 3.1 and 20 mg/kg bw per day) for 52 weeks. One female at 120 ppm died unexpectedly during week 49; the cause of death was not determined. No treatment-related clinical signs, alterations in body weight or food consumption, changes in ophthalmic parameters or findings at gross necropsy were reported.

The primary treatment-related effect observed was inhibition of cholinesterase activity in brain and erythrocytes (Table 2). Relative to the values for controls, brain acetylcholinesterase activity was significantly inhibited in all groups of males (by 17%, 53% and 66%, respectively) and in females at 120 and 800 ppm (by 49% and 66%, respectively), but was not significantly inhibited (by 11%) at 10 ppm. Erythrocyte cholinesterase activity was slightly increased in dogs of each sex at 10 ppm and significantly inhibited in dogs at 120 ppm (42–55%) and 800 ppm (76–87%). Despite severe inhibition of brain cholinesterase activity in both sexes at the two higher concentrations, the clinical signs usually associated with cholinesterase inhibition were not observed.

The NOAEL was 10 ppm (equal to 0.27 mg/kg bw per day) in male dogs on the basis of inhibition of brain and erythrocyte cholinesterase activity by more than 20% at 120 ppm (Dalgard, 1991, summarized from the evaluation made by JMPR in 2002).

Table 2. Mean percentage inhibition of acetylcholinesterase activity in dogs given diets containing acephate for 52 weeks

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

From Dalgard (1991)

2.3 Special studies

(a) Studies on inhibition of cholinesterase activity in vitro

The inhibitory activity (IC_{50}) of acephate technical on brain and erythrocyte acetylcholinesterase and plasma cholinesterase in vitro was determined for rats, monkeys and humans. The data are reported in Table 3 (Bennett & Morimoto, 1982, summarized from the evaluation made by JMPR in 2002).

(b) Short-term study of neurotoxicity

Rats

In the new short-term study of neurotoxicity that complied with the principles of GLP, groups of 12 male and 12 female Sprague-Dawley Crl:CD®(SD)IGS BR rats were fed diets containing acephate technical (purity, 99.1%) at a concentration of 0, 50, 100, 250, 500, 700 or 1000 ppm (equal to 0, 3.4, 6.7, 17.6, 36.5, 50.8 and 74.2 mg/kg bw per day for males, and 0, 3.8, 7.5, 19.3, 40.9, 57.2 and 89.7 mg/kg bw per day for females) for 49 days.

All animals survived to the scheduled termination and no treatment-related clinical or behavioural signs or observations at necropsy were reported at dietary concentrations of up to and including 1000 ppm.

Mean body-weight gains in male rats were significantly reduced at and above 250 ppm during the first 8 days of treatment**.** In the group receiving acephate technical at 1000 ppm, male body weights were consistently reduced after day 8 of the study. At termination, the mean body weight in this group was 11% lower than that of the controls. Consistent with these results, absolute feed consumption was decreased in male rats at 1000 ppm, compared with the controls. The values for relative food consumption were higher in rats receiving acephate at 250 to 1000 ppm than in the controls. In contrast, mean body weights and body-weight gains in the females were unaffected by administration of acephate technical at dietary concentrations of up to 1000 ppm. Compared with the controls, values for absolute and relative feed consumption in females were increased at 500, 700 and 1000 ppm.

There were no treatment-related changes in clinical observations, behaviour, motor activity, brain weight or in parameters included in the comprehensive functional observational battery (FOB) evaluations, even at the highest dose (1000 ppm). Tests conducted included, but were not limited to, observations and/or measurements of general appearance, behaviour (grooming, head shaking, vocalization, backward motion), body position and posture (e.g. hunchback posture), autonomic nervous system function (lachrymation, pupil diameter, respiration, excretion), motor coordination and activity, ambulation (gait abnormalities), response to handling or environmental stimulation or neural effects (tremor, convulsion, muscular contraction), reactivity and sensitivity (sensory motor responses to visual, auditory, tactile and painful stimuli), visual placing response and landing foot splay (gait and sensory motor coordination), and forelimb and hindlimb grip strength.

Table 3. Inhibition of cholinesterase activity in vitro by acephate in rats, monkeys and humans

Species	IC_{50} (mol/l)		
	Brain	Erythrocyte	Plasma
Rat	1.6×10^{-3}	1.3×10^{-3}	4.5×10^{-3}
Monkey	3.4×10^{-3}	2.7×10^{-3}	2.3×10^{-3}
Human	5.4×10^{-3}	2.7×10^{-3}	1.8×10^{-3}

From Bennett & Morimoto (1982) summarized from 2002 JMPR.

IC_{50}, concentration at which 50% of the enzymatic activity is inhibited.

Cholinesterase activity was measured in plasma, erythrocytes and the brain (results are given in Table 4). At lower concentrations (i.e. 50 or 100 ppm), cholinesterase activity was inhibited to a greater degree in the brain than in erythrocytes or plasma. At higher concentrations, the inhibition of cholinesterase activity in erythrocytes and the brain was similar.

In all exposure groups there was a reduction of more than 20% in the activity of brain, erythrocyte and plasma cholinesterase, except for erythrocyte cholinesterase in males at 50 ppm and plasma cholinesterase in females at 50 and 100 ppm. Cholinesterase activity was reduced in a dose-dependent manner in both sexes and the reduction in brain cholinesterase activity (ranging from 40% to 80%) was substantial.

No clinical or neurobehavioural effects were observed at any dose, even at the highest dose (1000 ppm), which caused an inhibition in brain cholinesterase activity of about 80%. No NOAEL could be identified in this study, the lowest-observed-adverse-effect level (LOAEL) being 50 ppm (equal to 3.4 mg/kg bw per day) on the basis of inhibition of brain cholinesterase activity (Foss, 2004).

Monkeys

Two male and two female cynomolgus monkeys *(Macaca fascicularis)* received technical acephate (purity, 98.7%) at a dose of 0 or 2.5 mg/kg bw per day by oral gavage for up to 34 consecutive days (33 days for males, 34 days for females).

No noteworthy differences between groups were observed with respect to clinical signs, food and water consumption, body-weight changes, physical examination, haematology, blood chemistry (except cholinesterase activity), urine analysis, organ weights or macroscopic pathology at terminal autopsy.

Cholinesterase activities were assayed in erythrocytes and plasma every 2 days and in brain at sacrifice. Erythrocyte acetyl- and plasma acetyl- and butyrylcholinesterase activities were lower in all treated monkeys than in controls. Maximum inhibition was observed after approximately 6 days for plasma cholinesterase and after 14 days for erythrocyte cholinesterase.

Mean inhibition (relative to mean pretreatment values) of 42% (males) and 43% (females) was recorded for plasma acetylcholinesterase activity and 37% (males) and 40% (females) for plasma butyrylcholinesterase activity during days 6–34. Mean inhibition of 53% (males) and 47% (females) for erythrocyte acetylcholinesterase activity was recorded during days 14–34. No erythrocyte butyrylcholinesterase activity was detected in treated or control animals. There was no relevant difference in the pattern of inhibition of plasma acetyl and butyryl activity or between sexes for each cholinesterase parameter.

Table 4. Inhibition of cholinesterase activity (per cent reduction relative to controls) in rats treated with acephate technical for 49 days

Substrate	Dietary concentration (ppm)					
	50	100	250	500	700	1000
Males						
Brain	–46.8	–50.9	–68.2	–68.2	–75.6	–79.3
Plasma	–21.6	–27.7	–37.4	–44.1	–53.1	–60.7
Erythrocyte	–2.7	–39.2	–63.5	–52.0	–86.1	–60.3
Females						
Brain	–39.6	–51.3	–61.7	–71.1	–69.7	–76.5
Plasma	–13.5	–17.6	–42.2	–52.0	–46.8	–71.9
Erythrocyte	–29.8	–38.3	–67.0	–81.3	–73.0	> –81.3

From Foss (2004)

Brain cholinesterase activity after 4 weeks of treatment was lower in all treated animals than in the controls. Compared with contemporaneous control values, treated animals showed a mean inhibition of 16% (males) and 32% (females) for butyrylcholinesterase activity and 50% (males) and 43% (females) for acetylcholinesterase activity. Thus, inhibition of brain cholinesterase activity was similar to that of acetylcholinesterase or less marked than that of butyrylcholinesterase. These levels of cholinesterase inhibition were without any visible cholinergic signs (Cummins, 1983, summarized from 1984 JMPR).

(c) Developmental neurotoxicity

In a new study of developmental neurotoxicity, groups of 25 presumed pregnant Crl:CD®(SD)IGS BR VAF/Plus® rats were given acephate (purity, 99.2%) at a dose of 0, 0.5, 1 or 10 mg/kg bw per day (dose volume, 10 ml/kg) by oral gavage, beginning on day 6 of gestation and continuing until day 6 of lactation. Rats in the F_1 generation were given acephate at the same doses via oral gavage from postnatal day 7 until weaning at postnatal day 21. At postnatal day 4, litters were standardized (reduced to five males and five females, when possible) and male and female pups were assigned to subsets for evaluation of neuromorphometry and neurohistology, water maze and passive avoidance behaviour, motor activity and auditory startle habituation at several time-points up to postnatal day 70. Pups were also evaluated for signs of autonomic dysfunction, and abnormal or unusual appearance, posture, movement and behaviour patterns. Plasma, erythrocyte and brain cholinesterase activities were measured at postnatal days 4 and 21. All female rats in the F_0 generation survived to scheduled sacrifice and no adverse treatment-related clinical observations occurred during gestation or lactation. Body weights, body-weight gains, and absolute and relative feed consumption were the same in all treatment groups throughout the study. There were no unusual necropsy observations. All pregnant rats delivered litters naturally and duration of gestation, number of implantation sites per delivered litter, gestation index and numbers of dams with stillborn pups were comparable between groups. Viability index, number of surviving pups per litter, and size of live litter were unaffected by exposure of the dams to acephate at a dose of up to 10 mg/kg per day. No clinical observations in the F_1 generation pups were attributable to exposure to acephate at a dose as high as 10 mg/kg bw per day and the few deaths that occurred during the postnatal period were not considered to be treatment-related because no dose-dependence was evident. Body weights, body-weight gains, feed consumption values, sexual maturation, memory and learning, motor activity, and acoustic startle habituation were similarly unaffected by exposure to acephate at doses of up to and including 10 mg/kg bw per day. Terminal body weights and brain weights as well as neuromorphometric and neurohistopathological parameters at postnatal day 21 and postnatal day 70 were not affected by treatment with acephate at any dose tested.

Cholinesterase activity in brain, erythrocytes and plasma was assayed on postnatal days 4 and 21 in pups. Neither consistent nor significant inhibition of plasma, erythrocyte or brain cholinesterase activity was observed in rats exposed from postnatal day 4. Administration of acephate to F_1 generation pups from postnatal days 7–21 resulted in significant dose-related reductions in brain and erythrocyte cholinesterase activity at postnatal day 21 at all doses (Table 5).

A NOAEL for brain cholinesterase inhibition could not be identified in this study, but no effects on functional development caused by neurotoxicity were observed (Hoberman, 2003).

The Meeting concluded that, while giving useful information, this study cannot be used for a quantitative risk assessment because repeated administration by gavage is not relevant for long-term exposure to acephate.

Table 5. Inhibition of cholinesterase activity (per cent reduction relative to controls) in rats treated with acephate from day 6 of gestation to postnatal day 21

Substrate	Dose (mg/kg bw per day)							
	Males				Females			
	0	0.5	1	10	0	0.5	1	10
Brain	0.0	–28.7	–33.7	–62.2	0.0	–25.4	–25.8	–57.9
Plasma	0.0	–4.8	–2.0	–46.3	0.0	–22.5	–15.8	–43.2
Erythrocytes	0.0	–22.7	–15.5	–50.4	0.0	–14.9	–19.2	–62.9

From Hoberman (2003)

* Significantly different from controls ($p < 0.05$)

** Significantly different from controls ($p < 0.01$)

3. Observations in humans

Groups of seven subjects received a single oral dose of acephate (purity, 99%) and three received lactose (placebo) in a hard gelatin capsule daily for 14 days. Groups of men received acephate at a dose of 0.35, 0.7, 1 or 1.2 mg/kg bw, and one group of women received acephate at a dose of 1 mg/kg bw. The volunteers remained at the clinic for 72 h after dosing. Vital signs were monitored at various times after dosing, and electrocardiograms (ECG), haematology, clinical chemistry and urine analysis were performed. The pharmacokinetics of acephate and its primary metabolite, methamidophos, was determined at several times. Blood samples were collected 1, 2, 4, 8, 12, 24, 48 and 72 h and 7 and 14 days after dosing. Urine samples were collected for 12 h before dosing and for 0–12 h, 12–24 h and 24–48 h after dosing. The subjects were monitored for adverse events throughout the study.

No difference in pharmacokinetics was seen between men and women, the T_{max} being 1–4 h. The concentration of acephate in the plasma of individuals in all treated groups was < 6% of the C_{max} by 24 h, and no acephate was detectable in plasma by 48 h. The terminal elimination half-life for acephate was between 3.5 h and 6.6 h for all subjects. The concentration of methamidophos was not quantifiable in the plasma of any subject given acephate at a dose of 0.35 mg/kg bw, but increased in a dose-related fashion in the other treated groups. The T_{max} for acephate was 1–4 h. The concentration of methamidophos in plasma was greatly reduced by 24 h after dosing, and the terminal elimination half-life was between 3.5 h and 12 h for all subjects. The recovery of test material (acephate plus acephate equivalents from methamidophos) in the urine, as measured during the 48 h after dosing, represented 26–62% of the administered dose in males and 12–53% in females. Most of the recovered acephate and methamidophos was found in the 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. During the first 12 h after dosing with acephate at 1 mg/kg bw, 35 000 and 31 000 ng/l of acephate and 300 and 280 ng/l of methamidophos were found in the urine of men and women, respectively. The fate of the other 50% or more of the administered dose is unknown, but may be mainly accounted for by incomplete gastrointestinal absorption.

Administration of doses of up to 1.2 mg/kg bw to men and 1.0 mg/kg bw to women did not cause any clinically significant changes in vital signs, ECGs or haematological, clinical chemical or urinary parameters or the results of physical examinations. Administration of acephate at the highest dose resulted in a statistically significant reduction in mean plasma cholinesterase activity in men, with decreases of 13%, 8.9%, 9.1% and 8.6% from the baseline value 12, 24, 48 and 72 h after dosing, respectively. A statistically significant reduction in mean erythrocyte cholinesterase activity was found 12 h after dosing in men (6.7% change from baseline), the only dose and time at which both plasma and erythrocyte cholinesterase activity were similarly affected in men. No

significant effects on cholinesterase activity were found at lower doses. Statistical analysis of the results for women given acephate at 1 mg/kg bw showed a significant reduction in mean plasma cholinesterase activity at 8, 12 and 24 h (by 13%, 12% and 10% from baseline) and a significant reduction in mean erythrocyte cholinesterase activity 12 h after dosing only after exclusion of outliers (11% change from baseline). None of the other comparisons was statistically significant.

The results indicated that erythrocyte cholinesterase activity is not inhibited at 1 mg/kg bw in women and 1.2 mg/kg bw in men, the highest doses tested (Freestone & McFarlane, 2000, summarized from 2002 JMPR).

Systemic exposure to acephate and methamidophos in humans was evaluated using the results of the above study, in which volunteers received a single oral dose of acephate (Freestone & McFarlane, 2000, summarized from 2002 JMPR). The results for pharmacokinetics indicated that the plasma concentrations of acephate and its primary metabolite methamidophos after repeated daily intake of acephate could be predicted. The underlying concept is that the exposure–time profiles for repeated doses with a fixed interval between doses and plasma concentration–time profiles based on complete elimination after each single dose are expected to be additive. As the plasma half-life determined within 0–24 h was 4.3–6.6 h, accumulation of acephate was negligible. Thus, the predicted plasma concentrations after infinite doses of acephate are comparable with that observed after a single dose, owing to the relatively short half-life of acephate. The situation for methamidophos is similar.

In conclusion, the predicted systemic concentrations of both acephate and methamidophos after infinite, repeated daily doses of acephate might be comparable to the actual measured systemic concentration after a single oral dose. If it is assumed that adverse effects are a monotonic function of systemic exposure to acephate and methamidophos and that these concentration–response relationships do not change with time, the NOAELs after a single oral dose might also apply to repeated daily intake of acephate (Tozer, 2000, summarized from 2002 JMPR).

In a new study (which complied with GLP and ethical guidelines) to evaluate the effect of acephate on erythrocyte and plasma cholinesterase in human volunteers, ten men received acephate technical as single doses at 0.25 mg/kg bw per day and five men were given placebo (lactose) daily for 28 consecutive days.

Volunteers underwent medical/clinical screening during the week before the start of dosing and were resident at the clinic throughout the 28-day dosing period; they returned for a follow-up visit on day 35. Possible adverse clinical signs and symptoms that were recorded included possibly cholinergic as well as non-cholinergic effects, such as dyspnoea, nasal congestion, nasal passage irritation, pharyngolaryngeal pain, reversible airway obstruction, rhinitis, sinus pain, sneezing, skin irritation, dizziness, headache, paraesthaesia, somnolence, facial pain, musculo-skeletal pain, nasopharyngitis, feeling cold, abdominal pain, sinus tachycardia, and neutrophilia.

None of these signs was treatment-related. One subject was withdrawn from the study because of reversible airway obstruction that resulted in hospitalization. Monitoring of changes in the results of haematology, clinical chemistry, urine analysis, vital signs, ECG, and physical examination at various time-points throughout the study was carried out.

There were no treatment-related changes from baseline for any haematology, clinical chemistry, ECG or urine analysis parameter and no changes in vital signs or physical examination

For cholinergic parameters, blood samples for measurement of erythrocyte and plasma cholinesterase activity were collected on days 7, 4, 2 and 1 before dosing, 1, 2, 4, 8 and 12 h after dosing on day 1, and again before dosing and 4 h after dosing on days 2, 3, 5, 7, 10, 14, 17, 21, 24 and 28. Cholinesterase activities were measured during the 28-day dosing period and were compared against the corresponding baseline levels before dosing using a repeated measures analysis of variance (ANOVA). Pair-wise comparisons of cholinesterase activity after administration of placebo or acephate technical were also made at each time-point. None of the

differences in mean percentage change from baseline plasma cholinesterase activity observed for the groups receiving placebo or acephate were statistically significant at the 5% level. Values for plasma cholinesterase activity over the first 24 days of exposure were below baseline levels for groups treated with placebo or with acephate. The maximum mean decreases in plasma cholinesterase activity were 7.5% on day 1 (8 h) in the group receiving placebo and 7.8% on day 21 (4 h) in the group receiving acephate. Although data on erythrocyte cholinesterase activity were more variable than those for plasma cholinesterase, there was no indication of any decrease from baseline erythrocyte cholinesterase values after administration of either placebo or acephate technical during the 35 days of the study. After the first 2 days of the study, when they were slightly below baseline levels, erythrocyte cholinesterase values were typically above baseline. Maximum mean decreases were 10.6% on day 1 (8 h) in the group receiving placebo and 10.2% on day 2 (before dose) in the group treated with acephate.

The NOAEL for the clinical signs and erythrocyte and plasma cholinesterase inhibition was 0.25 mg/kg per day, the only dose tested (Freestone & Jackson, 2003).

Comments

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 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 ≤ 5% was methamidophos. Small amounts of *O*-desmethyl acephate, *O*-desmethyl methamidophos and *O,S*-dimethyl phosphorothioate, were also identified in the urine.

The pharmacokinetics of acephate were 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 6. There were no relevant differences between humans and rats, considering the different methods used.

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 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.

Table 6. 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} (μg/ml)	0.45	0.8	1.35	1.6	23	90

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 2.

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 pretreatment 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 day 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 to1.2 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, ECG 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 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).

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Rat	Acute neurotoxicity[a,b]	Toxicity	2.5 mg/kg bw	5 mg/kg bw
	Short-term study of neurotoxicity[c]	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[a]	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[c]	Toxicity	10 ppm, equal to 0.27 mg/kg bw per day[d]	120 ppm, equal to 3.1 mg/kg bw per day
Human	Single-dose study[e]	Toxicity	1.2 mg/kg bw[f]	—
	28-day study[e]	Toxicity	0.25 mg/kg bw per day[f]	—

[a] Gavage administration

[b] Tested only in females

[c] Dietary administration

[d] Marginal effects on brain acetylcholinesterase activity, of equivocal toxicological relevance

[e] Oral administration

[f] 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

Absorption, distribution, excretion and metabolism in mammals	
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
Acute toxicity	
Rat LD_{50} oral	1000 mg/kg bw
Rabbit LD_{50} dermal	> 2000 mg/kg bw
Rat LC_{50} 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)
Short-term studies of toxicity	
Target/critical effect	Nervous system/inhibition of cholinesterase activity
Lowest relevant oral NOAEL[a]	10 ppm, equal to 0.58 mg/kg bw per day (13-week study in rats)
Genotoxicity	
	Unlikely to be genotoxic in vivo
Long-term studies of toxicity and carcinogenicity	
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
Reproductive toxicity	
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)

Neurotoxicity/delayed neurotoxicity	
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)
Other toxicological studies	
	Toxicokinetic and metabolism data not significantly different from data in rats

Summary	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

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