5.10 ETHEPHON (106)

TOXICOLOGY

Ethephon is the ISO-approved common name for 2-chloroethylphosphonic acid (IUPAC), which has the CAS number 16672-87-0. Ethephon is a plant growth regulator that acts by release of ethylene, directly influencing several physiological processes, such as ripening and maturation, and stimulating the production of endogenous ethylene. Ethephon is used on a variety of crops, including fruits, vegetables, cereals and oilseed crops.

Ethephon was previously evaluated for toxicology by JMPR in 1977, 1978, 1993, 1995, 1997 and 2002. In 1993, the Meeting established an ADI of 0–0.05 mg/kg bw on the basis of a NOAEL of 0.5 mg/kg bw per day in studies in humans given repeated ethephon doses and application of a 10-fold safety factor. In 2002, the Meeting established an ARfD of 0.05 mg/kg bw on the basis of human data.

Ethephon was re-evaluated by the present Meeting as part of the periodic review programme of CCPR. Both new toxicity studies with ethephon in dogs and with the ethephon metabolite 2-hydroxyethyl phosphonic acid (or 2-hydroxyethephon; HEPA) in rats and previously submitted studies were considered by the present Meeting.

Some of the critical studies do not comply with GLP, as the data were generated before the implementation of GLP regulations. Overall, however, the Meeting considered that the database was adequate for the risk assessment.

Available studies with ethephon in humans were performed in accordance with the ethical standards at the time and were compliant with the Declaration of Helsinki.

Biochemical aspects

In rats, absorption of ethephon was rapid, with a time to reach the maximum plasma concentration (T_{max}) of 1.0–1.3 hours after a single oral dose of 50 mg/kg bw and 1.9–2.5 hours after a single oral dose of 1000 mg/kg bw. Peak plasma concentrations at 1000 mg/kg bw were less than proportional to dose, compared with those after 50 mg/kg bw. Six days after a single dose, tissues and carcass contained at most 0.06% of the administered radioactivity. Highest concentrations were found in bone, liver, blood and kidney. Radioactivity concentrations in brain were low. Radioactivity was excreted in urine (47–60%), expired air (18–22%, mainly ethylene) and faeces (4–6.5%), indicating that at least 65% of the administered dose was absorbed. Excretion was largely complete within the first 24 hours after dose administration. Ethephon was mainly recovered as its monosodium and disodium salts, ethylene and, to a lesser extent, HEPA. There were no remarkable differences in absorption and excretion between sexes and between oral dosing regimens.

Ethephon inhibits butyrylcholinesterase (BuChE) activity in plasma and, to a lesser extent, acetylcholinesterase (AChE) activity in erythrocytes. Ethephon has virtually no effect on brain AChE activity in vivo. In vitro studies showed that BuChE in plasma of dog, human and mouse was more sensitive to ethephon inhibition than BuChE in plasma of rabbit, rat, chicken and guinea-pig. Mechanistic investigations indicate that ethephon inhibits BuChE activity by phosphorylation at Ser-198 of the esteratic site, leading to the formation of a phosphobutyrylcholinesterase.

Toxicological data

The acute toxicity of ethephon is low (rat oral LD_{50} = 1564 mg/kg bw; rabbit dermal LD_{50} = 983 mg/kg bw; rat inhalation LC_{50} = 3.26 mg/L). Ethephon was severely irritating to the skin of rabbits. No eye irritation study was required, as technical ethephon has a pH of less than 2 and is therefore assumed to be corrosive to the eye. Ethephon was not a skin sensitizer in a Magnusson and Kligman test in guinea-pigs.

In repeated-dose oral toxicity studies with ethephon in mice, rats and dogs, the main effect was reduction of erythrocyte AChE activity.

In a 28-day study in mice administered ethephon at a dietary concentration of 0, 30, 100, 300, 1000 or 3000 ppm (equal to 0, 5.3, 18, 51, 181 and 546 mg/kg bw per day for males and 0, 6.5, 22, 69, 210 and 635 mg/kg bw per day for females, respectively), the NOAEL was 100 ppm (equal to 22 mg/kg bw per day), based on reduction of erythrocyte AChE activity observed in females at 300 ppm (equal to 69 mg/kg bw per day). In a second 28-day study in mice administered ethephon at a dietary concentration of 0, 3000, 10 000, 25 000 or 50 000 ppm (equal to 0, 530, 1800, 4500 and 10,000 mg/kg bw per day for males and 0, 630, 2200, 5900 and 15 000 mg/kg bw per day for females, respectively), no NOAEL could be identified, as reductions in erythrocyte AChE activity were observed at all doses.

In a 28-day range-finding study in rats administered ethephon at a dietary concentration of 0, 625, 1250, 2500, 5000 or 10 000 ppm (equal to 0, 52, 106, 214, 431 and 831 mg/kg bw per day for males and 0, 59, 120, 251, 487 and 980 mg/kg bw per day for females, respectively), the NOAEL was 625 ppm (equal to 52 mg/kg bw per day), based on reduction of erythrocyte AChE activity observed in males at 1250 ppm (equal to 106 mg/kg bw per day). In a second 28-day range-finding study in rats administered ethephon at a dietary concentration of 0, 10 000, 25 000 or 50 000 ppm (equal to 0, 962, 2300 and 4673 mg/kg bw per day for males and 0, 996, 2488 and 4900 mg/kg bw per day for females, respectively), no NOAEL could be identified, as reduction of AChE activity in erythrocytes was observed at all doses.

In a 1-year study in dogs administered ethephon at a dietary concentration of 0, 100, 300, 1000 or 2000 ppm (equal to 0, 2.8, 8.1, 27 and 54 mg/kg bw per day for males and 0, 2.6, 8.4, 30 and 50 mg/kg bw per day for females, respectively), the NOAEL was 1000 ppm (equal to 27 mg/kg bw per day), based on a lower body weight gain at 52 weeks in both sexes and low absolute and relative spleen weights in males at 2000 ppm (equal to 54 mg/kg bw per day). The effect of ethephon treatment on cholinesterase activity was not assessed in this study.

In a 2-year study in dogs administered ethephon at a dietary concentration of 0, 30, 300 or 1500 ppm (equal to 0, 0.86, 7.6 and 42.2 mg/kg bw per day for males and 0, 0.86, 8.4 and 47.8 mg/kg bw per day for females, respectively), the NOAEL was 30 ppm (equal to 0.86 mg/kg bw per day), based on reduction of erythrocyte AChE activity at 300 ppm (equal to 7.6 mg/kg bw per day).

In a 78-week carcinogenicity study in mice administered ethephon at a dietary concentration of 0, 30, 300 or 1000 ppm (equivalent to 0, 4.5, 45 and 150 mg/kg bw per day, respectively), the NOAEL was 30 ppm (equivalent to 4.5 mg/kg bw per day), based on reduction of erythrocyte AChE activity observed at weeks 52 and 78 in females at 300 ppm (equivalent to 45 mg/kg bw per day). No treatment-related tumours were observed in mice in this study.

In a second 78-week carcinogenicity study in which mice were administered ethephon at a dietary concentration of 0, 100, 1000 or 10 000 ppm (equal to 0, 14, 139 and 1477 mg/kg bw per day for males and 0, 17, 173 and 1782 mg/kg bw per day for females, respectively), the NOAEL was 100 ppm (equal to 14 mg/kg bw per day), based on reduction of erythrocyte AChE activity in both sexes at 1000 ppm (equal to 139 mg/kg bw per day). No treatment-related tumours were observed in mice in this study.

The overall NOAEL for the two 78-week studies in mice was 100 ppm (equal to 14 mg/kg bw per day). The overall LOAEL was 300 ppm (equivalent to 45 mg/kg bw per day).

In a 2-year toxicity and carcinogenicity study in rats administered ethephon at a dietary concentration of 0, 30, 300 or 3000 ppm (equal to 0, 1.2, 13 and 129 mg/kg bw per day for males and 0, 1.6, 16 and 171 mg/kg bw per day for females, respectively), the NOAEL was 300 ppm (equal to 13 mg/kg bw per day), based on reduction of erythrocyte AChE activity in both sexes at 3000 ppm (equal to 129 mg/kg bw per day). No treatment-related tumours were observed in rats in this study.

In a second 2-year toxicity and carcinogenicity study in which rats were administered ethephon at a dietary concentration of 0, 300, 3000, 10 000 or 30 000 ppm (equal to 0, 13, 131, 446 and 1416 mg/kg bw per day for males and 0, 16, 161, 543 and 1794 mg/kg bw per day for females, respectively), the NOAEL was 300 ppm (equal to 13 mg/kg bw per day), based on reduction of

erythrocyte AChE activity observed in both sexes at 3000 ppm (equal to 131 mg/kg bw per day). No treatment-related tumours were observed in rats in this study.

The overall NOAEL for the two 2-year studies in rats was 300 ppm (equal to 13 mg/kg bw per day). The overall LOAEL was 3000 ppm (equal to 129 mg/kg bw per day).

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

Ethephon was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. There was no evidence of genotoxicity in vitro, except for a positive response in *Salmonella typhimurium* strain TA1535 in both the absence and presence of metabolic activation. There was no evidence of genotoxicity in vivo.

Based on the weight of evidence, the Meeting concluded that ethephon is unlikely to be genotoxic in vivo.

In view of the lack of genotoxicity in vivo and the absence of carcinogenicity in mice and rats, the Meeting concluded that ethephon is unlikely to pose a carcinogenic risk to humans.

In a two-generation reproductive toxicity study in rats administered ethephon at a dietary concentration of 0, 300, 3000 or 30 000 ppm (equal to 0, 22, 220 and 2260 mg/kg bw per day for F_0 males and 0, 25, 260 and 2570 mg/kg bw per day for F_0 females, respectively; and 0, 20, 200 and 2220 mg/kg bw per day for F_{1b} males and 0, 24, 245 and 2520 mg/kg bw per day for F_{1b} females, respectively), the NOAEL for parental toxicity was 300 ppm (equal to 20 mg/kg bw per day), based on an increased incidence of loose faeces in F_{1b} males at 3000 ppm (equal to 200 mg/kg bw per day). The NOAEL for offspring toxicity was 300 ppm (equal to 22 mg/kg bw per day), based on an increased mortality in F_{1b} pups from PND 4 to PND 7 and a reduction in body weight gain during lactation in F_{2b} pups at 3000 ppm (equal to 220 mg/kg bw per day). The NOAEL for reproductive toxicity was 30 000 ppm (equal to 2220 mg/kg bw per day), the highest dose tested. The effect of ethephon treatment on cholinesterase activity was not assessed in this study.

In a developmental toxicity study in rats administered ethephon by gavage at a dose of 0, 200, 600 or 1800 mg/kg bw per day, the NOAEL for maternal toxicity was 600 mg/kg bw per day, based on increased mortality, clinical signs (salivation), reduced body weight gain, and various macroscopic findings and histological changes (focal lymphoid hyperplasia of the spleen and focal parenchymal fibrosis of the liver) at 1800 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 1800 mg/kg bw per day, the highest dose tested. The effect of ethephon treatment on cholinesterase activity was not assessed in this study.

In a second developmental toxicity study in rats administered ethephon by gavage at a dose of 0, 125, 250 or 500 mg/kg bw per day, the NOAEL for maternal toxicity and for embryo and fetal toxicity was 500 mg/kg bw per day, the highest dose tested. The effect of ethephon treatment on cholinesterase activity was not assessed in this study.

In a developmental toxicity study in rabbits administered ethephon by gavage at a dose of 0, 50, 100 or 250 mg/kg bw per day, the NOAEL for maternal toxicity was 50 mg/kg bw per day, based on a body weight reduction from GD 7 to GD 12 and an increased number of resorptions at 100 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 50 mg/kg bw per day, based on a reduced number of live fetuses and reduced viability of fetuses at 100 mg/kg bw per day. The effect of ethephon treatment on cholinesterase activity was not assessed in this study.

In a second developmental toxicity study in rabbits administered ethephon by gavage at a dose of 0, 62.5, 125 or 250 mg/kg bw per day, the NOAEL for maternal toxicity was 125 mg/kg bw per day, based on mortality, clinical signs of toxicity and decreased body weight at 250 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 125 mg/kg bw per day. As three does died and 14 does were killed in a moribund condition at 181 mg/kg bw per day, the number of fetuses in the high-dose group was insufficient to conclude on the effects of ethephon on prenatal development at 250 mg/kg bw per day. The effect of ethephon treatment on cholinesterase activity was not assessed in this study.

In a pilot neurotoxicity study in rats aimed at finding the time to peak effect after a single gavage dose of ethephon of 0, 250, 500, 1000 or 2000 mg/kg bw, the maximum suppression of plasma cholinesterase activity for all groups occurred at 4–8 hours following treatment. Erythrocyte and brain AChE levels were not affected by treatment in this study.

In an acute neurotoxicity study in rats administered ethephon by gavage at a dose of 0, 500, 1000 or 2000 mg/kg bw, no NOAEL could be identified, as increased incidences of myosis were observed at all dose levels. The effect of ethephon treatment on cholinesterase activity was not assessed in this study.

In a 13-week neurotoxicity study in rats administered ethephon by gavage at a dose of 0, 75, 150 or 400 mg/kg bw per day (the high dose was decreased to 300 mg/kg bw per day during week 10/11 of treatment), the NOAEL was 75 mg/kg bw per day, based on reduction of erythrocyte cholinesterase in females at 150 mg/kg bw per day.

In a 28-day neurotoxicity study in dogs administered ethephon at a dietary concentration of 0, 250 or 750 ppm (equal to 0, 6 and 14 mg/kg bw per day, respectively), the NOAEL was 250 ppm (equal to 6 mg/kg bw per day), based on reduction of AChE activity in erythrocytes at 750 ppm (equal to 14 mg/kg bw per day).

In a 90-day neurotoxicity study in dogs administered ethephon at a dietary concentration of 0, 70, 140 or 525 ppm (equal to 0, 2, 4 and 15 mg/kg bw per day for males and 0, 2, 4 and 18 mg/kg bw per day for females, respectively), the NOAEL was 70 ppm (equal to 2 mg/kg bw per day), based on reduction of AChE activity in erythrocytes at 140 ppm (equal to 4 mg/kg bw per day).

The Meeting noted that in the neurotoxicity studies, no clinical signs of neurotoxicity were observed, even though erythrocyte AChE activity was reduced.

No evidence for delayed neurotoxicity was observed in three studies in chickens.

Toxicological data on metabolites and/or degradates

HEPA is a significant metabolite of ethephon in rats and is also the main plant metabolite. Acute and short-term toxicity and genotoxicity studies with HEPA were available. The acute oral toxicity of HEPA was low (rat LD_{50} > 2000 mg/kg bw). HEPA did not cause inhibition of plasma cholinesterase activity in vitro.

In a 28-day toxicity study in rats administered HEPA by gavage at a dose of 0, 125, 350 or 1000/700 mg/kg bw per day (the highest dose was reduced from 1000 to 700 mg/kg bw per day from day 5 onwards, as a result of mortality), the NOAEL was 350 mg/kg bw per day, based on mortality, clinical signs, reduced body weight gain and feed consumption (females only), changes in urinary parameters, and various macroscopic findings and histological changes (epithelial necrosis and intraluminal inflammatory exudates in trachea) observed at 1000/700 mg/kg bw per day. The effects observed at the high dose are considered related to the gavage administration and the physicochemical properties of HEPA.

HEPA was negative in a gene mutation test in bacteria and in a gene mutation test and a chromosomal aberration test in mammalian cells in vitro.

In gavage studies in rats, the toxicity of HEPA was similar to that of ethephon. The effects observed in these studies with high doses of HEPA or ethephon are likely the result of a local gastrointestinal effect due to the physicochemical properties of these compounds and are therefore not relevant to the risk assessment. As HEPA does not reduce cholinesterase activity and as the NOAEL for HEPA in a 28-day gavage study is at least 2 orders of magnitude higher than the NOAEL of 0.5 mg/kg bw in humans that forms the basis of the ADI and ARfD, HEPA is not considered to be a toxicologically relevant metabolite.

Human data

In a 28-day study in human volunteers, five males and five females received ethephon at oral (capsule) doses of approximately 1.5 mg/kg bw per day for males and 2.2 mg/kg bw per day for

females, divided over three daily dosages. Three males and three females received placebo. Transient, subjective complaints, such as diarrhoea or urgency of bowel movements, were observed on 1–4 days in the first week of treatment in four volunteers receiving ethephon, but not in control subjects. Urgency or an increased frequency of urination was observed during the course of the study in one control and five treated volunteers. In addition, loose stools, stomach cramps and/or gas, flank pain, and loss or increase of appetite were occasionally reported by some volunteers treated with ethephon. No changes in plasma and erythrocyte cholinesterase activities and no persistent side-effects were observed. No treatment-related changes in haematology, clinical biochemistry or urine analysis parameters were noted.

In a 16-day study, volunteers received ethephon orally (by capsule) at a dose of 0 or 0.5 mg/kg bw per day (divided over three daily dosages). Ten males and 10 females received ethephon, and six males and four females received placebo. No treatment-related clinical signs or changes in erythrocyte AChE values or in haematology, clinical chemistry or urine analysis parameters were observed.

In a 22-day volunteer study using ethephon at oral (capsule) doses of 0 (three males and three females), 0.17 (three males and four females) and 0.33 mg/kg bw per day (four males and three females), no treatment-related clinical signs or changes in erythrocyte AChE activities or haematology, clinical chemistry or urine analysis parameters were observed.

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

Toxicological evaluation

The Meeting reaffirmed the ADI of 0–0.05 mg/kg bw, established on the basis of the overall NOAEL of 0.5 mg/kg bw per day in studies in humans, based on transient, subjective complaints, such as diarrhoea and urgency of bowel movements, loose stools, stomach cramps and/or gas, urgency or an increased frequency of urination, flank pain, and loss or increase of appetite, with the application of a 10-fold safety factor.

The Meeting reaffirmed the ARfD for ethephon of 0.05 mg/kg bw, established on the basis of the overall NOAEL of 0.5 mg/kg bw per day in studies in humans, based on transient, subjective complaints, such as diarrhoea and urgency of bowel movements, loose stools, stomach cramps and/or gas, urgency or an increased frequency of urination, flank pain, and loss or increase of appetite observed during the first week of treatment, with the application of a 10-fold safety factor.

Levels relevant to risk assessment of ethephon

Species	Study	Effect	NOAEL	LOAEL
Mouse	Seventy-eight-week studies of toxicity and carcinogenicity[a,b]	Toxicity	100 ppm, equal to 14 mg/kg bw per day	300 ppm, equivalent to 45 mg/kg bw per day
		Carcinogenicity	10 000 ppm, equal to 1 477 mg/kg bw per day[c]	–
Rat	Two-year studies of toxicity and carcinogenicity[a,b]	Toxicity	300 ppm, equal to 13 mg/kg bw per day	3 000 ppm, equal to 129 mg/kg bw per day
		Carcinogenicity	30 000 ppm, equal to 1 416 mg/kg bw per day[c]	–
	Two-generation study of reproductive	Reproductive toxicity	30 000 ppm, equal to 2 220 mg/kg bw per day[c]	–

Species	Study	Effect	NOAEL	LOAEL
	toxicity[a]	Parental toxicity	300 ppm, equal to 20 mg/kg bw per day	3 000 ppm, equal to 200 mg/kg bw per day
		Offspring toxicity	300 ppm, equal to 22 mg/kg bw per day	3 000 ppm, equal to 220 mg/kg bw per day
	Developmental toxicity study[d]	Maternal toxicity	600 mg/kg bw per day	1 800 mg/kg bw per day
		Embryo and fetal toxicity	1 800 mg/kg bw per day[c]	–
Rabbit	Developmental toxicity study[d]	Maternal toxicity	50 mg/kg bw per day	100 mg/kg bw per day
		Embryo and fetal toxicity	50 mg/kg bw per day	100 mg/kg bw per day
Dog	Thirteen-week study of neurotoxicity[a]	Toxicity	70 ppm, equal to 2 mg/kg bw per day	140 ppm, equivalent to 4 mg/kg bw per day
	Two-year study of toxicity[a]	Toxicity	30 ppm, equal to 0.86 mg/kg bw per day	300 ppm, equal to 7.6 mg/kg bw per day
Human	Sixteen- and 28-day studies of toxicity[b,e]	Toxicity	0.5 mg/kg bw per day	1.5 mg/kg bw per day

[a] Dietary administration.
[b] Two or more studies combined.
[c] Highest dose tested.
[d] Gavage administration.
[e] Capsule administration.

Estimate of acceptable daily intake (ADI)

0–0.05 mg/kg bw

Estimate of acute reference dose (ARfD)

0.05 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 exposure

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

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid; > 65% at 50 and 1 000 mg/kg bw (rat)
Dermal absorption	No data
Distribution	Widespread distribution, highest concentrations found in bone, liver, blood and kidney; low concentrations in brain (rat)
Potential for accumulation	Low

Rate and extent of excretion	Rapid; largely complete within the first 24 h after dose administration
Metabolism in animals	Converted to its monosodium and disodium salts, ethylene and, to a lesser extent, HEPA
Toxicologically significant compounds in animals and plants	Ethephon
Acute toxicity	
Rat, LD_{50}, oral	1 564 mg/kg bw
Rabbit, LD_{50}, dermal	983 mg/kg bw
Rat, LC_{50}, inhalation	3.26 mg/L
Rabbit, dermal irritation	Severely irritating
Rabbit, ocular irritation	Assumed to be corrosive, pH < 2
Guinea-pig, dermal sensitization	Not sensitizing (maximization test)
Short-term studies of toxicity	
Target/critical effect	Reduction of erythrocyte AChE activity
Lowest relevant oral NOAEL	0.86 mg/kg bw per day (dog)
Lowest relevant dermal NOAEL	237 mg/kg bw per day (highest dose tested); severe dermal irritation at 119 mg/kg bw per day (rabbit)
Lowest relevant inhalation NOAEC	No data
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Reduction of erythrocyte AChE activity
Lowest relevant NOAEL	13 mg/kg bw per day (rat)
Carcinogenicity	Not carcinogenic in mice or rats[a]
Genotoxicity	
	Unlikely to be genotoxic in vivo[a]
Reproductive toxicity	
Target/critical effect	No reproductive effect
Lowest relevant parental NOAEL	20 mg/kg bw per day (rat)
Lowest relevant offspring NOAEL	22 mg/kg bw per day (rat)
Lowest relevant reproductive NOAEL	2 220 mg/kg bw per day (highest dose tested; rat)
Developmental toxicity	
Target/critical effect	Reduced viability and number of live fetuses
Lowest relevant maternal NOAEL	50 mg/kg bw per day (rabbit)
Lowest relevant embryo/fetal NOAEL	50 mg/kg bw per day (rabbit)
Neurotoxicity	
Acute neurotoxicity LOAEL	500 mg/kg bw
Subchronic neurotoxicity NOAEL	2 mg/kg bw per day (dog)
Developmental neurotoxicity NOAEL	No data
Delayed neurotoxicity	Negative
Other toxicological studies	

Studies with HEPA	Oral LD_{50}: > 2 000 mg/kg bw (rat) 28-day study: NOAEL = 350 mg/kg bw per day (rat) Negative in a gene mutation test in bacteria and in a gene mutation test and a chromosomal aberration test in mammalian cells in vitro
Human data	
	NOAEL 0.5 mg/kg bw per day. Transient, subjective clinical signs were reported in a 28-day oral (capsule) study in human volunteers, using ethephon doses of approximately 1.5–2.2 mg/kg bw per day. No effects on plasma or erythrocyte cholinesterase activities.

[a] Unlikely to pose a carcinogenic risk to humans from the diet.

Summary

	Value	Study	Safety factor
ADI	0–0.05 mg/kg bw	Sixteen-day and 28-day studies in humans	10
ARfD	0.05 mg/kg bw	Sixteen-day and 28-day studies in humans	10

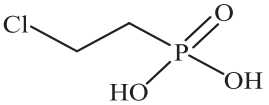
RESIDUE AND ANALYTICAL ASPECTS

Ethephon, 2-chloroethylphosphonic acid, is a systemic plant growth regulator belonging to the phosphonate family. It is readily absorbed by the plant and releases ethylene, a natural plant hormone. Ethylene not only influences directly several physiological processes such as ripening and maturation, but also stimulates the endogenous ethylene production. It has been registered in many countries for a variety of crops, including fruits, vegetables, cereals and oilseed crops.

Ethephon was first evaluated by JMPR in 1977 as a new compound, and then reviewed several times for residues. It was evaluated under the periodic review programme in 1994. The compound was listed in the Priority List by the Forty-sixth Session of CCPR in 2014 for toxicological and residue evaluation by the current Meeting in the CCPR periodic review programme.

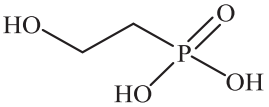
The Meeting received information on identity, metabolism and environmental fate, residue analysis, use pattern, supervised trials (on apples, cherries, grapes, figs, olives, pineapples, tomatoes, cereals, and cotton), processing, and animal feeding studies.

In this Appraisal, the following names were used for referred compounds.



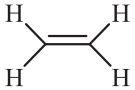
Ethephon

2-Chloroethylphosphonic acid



HEPA

(2-hydroxyethyl)-phosphonic acid



Ethylene

Plant metabolism

The Meeting received information on plant metabolism studies conducted on a variety of plants including information from the published scientific literature. The information dated from 1962 to 2003 and covered peaches, grapes, pineapples, cucumbers, squash, melons, tomatoes, wheat, hazelnuts, walnuts and cotton.

Many studies conducted on various plants indicate the release of ethylene after treatment with ethephon. In several of such studies, methanol, acidified methanol or water was used to extract ethephon from fruits and/or leaves and, where data are available, significant amount of the applied radioactivity (> 60%) or TRR (> 80%) was recovered in the surface wash and solvent extract combined.

The studies involving characterization and identification of other metabolites are described below.

Tomato plants grown outdoor were treated with a foliar spray of uniformly labelled [^{14}C]ethephon at a rate approximating 1.46 kg ai/ha at the "green mature" or "colour break" growth stage and fruits were harvested 0, 5 and 12 days after the treatment (DAT). The majority of the radioactivity was recovered from the methanol surface wash on 0 DAT but 96% (including surface wash) and 98% of the TRR was recovered in methanol extracts of 5 DAT and 12 DAT samples respectively.

The predominant radioactive residue in methanol extract of tomato fruit was ethephon, 70% and 59% of the TRR corresponding to 1.2 mg/kg and 0.68 mg/kg in 5 DAT and 12 DAT was found in fruits, respectively. The concentration of ethephon decreased over the time period in the study from 7.5 mg/kg at 0 DAT to 0.68 mg/kg at 12 DAT. The only significant metabolite found was HEPA accounting for 15% TRR (0.26 mg/kg) on 5 DAT and 13% TRR (0.15 mg/kg) on 12 DAT. No other metabolites exceeded 5% TRR in the methanol extract.

Wheat plants grown outdoor were treated with a foliar spray of [^{14}C]ethephon at a rate of 0.36 kg ai/ha and 3.6 kg ai/ha at the forage stage (BBCH 39) and forage samples were collected on 0 DAT, hay on 14 DAT and grain and straw on 34 DAT. The majority of radioactivity was recovered in methanol extracts of plant parts (hay and straw) on 14 and 34 DAT regardless of the dose used (94% TRR including 1% in surface wash in hay of both doses and 58% and 74% TRR in straw respectively) while radioactivity was similarly distributed in the methanol surface wash and methanol extract (45–46% and 54–55% TRR) of forage on 0 DAT. Unextracted residues were about 5% in 14 DAT for hay and 10% (1×) and 26% (10×) in 34 DAT for straw.

Methanol extraction recovered only 28 and 22% TRR from grain samples after the low and high doses. Acid hydrolysis of the remaining solid released a further 56 and 71% TRR; extraction of the post-hydrolysis solids released a total of 9.9% and 4.3% TRR, respectively. This indicates the presence of significant conjugates in grains. Unextracted residues were 1.8–6.0% TRR.

Most of the TRR was attributed to the sum of ethephon and HEPA. The major radioactive residue in 14 DAT hay was HEPA (72% TRR and 3.7 mg/kg) followed by ethephon (20% TRR and 1.0 mg/kg). In the 34 DAT straw, the major radioactive residue was ethephon (62% TRR and 1.5 mg/kg).

In 34 DAT grain, HEPA was found at a similar level as ethephon after the low dose (HEPA 48% TRR and 0.51 mg/kg and ethephon, 44% and 0.47 mg/kg). After the higher dose, approximately two times larger amount of HEPA was found than ethephon (HEPA, total of 60% TRR and 2.0 mg/kg; and ethephon, total of 32% TRR and 1.1 mg/kg). No other metabolites exceeded 3% of TRR.

Cotton plants grown outdoor were treated with a foliar spray at a rate of 1.4 kg ai/ha seven days before harvest. Plants were harvested at 7 DAT. The majority of radioactivity was recovered in methanol/water (9:1) for gin trash (89% TRR) and in methanol extract for seeds (82% TRR).

The predominant radioactive residue in gin trash was ethephon at 93% TRR and 30 mg/kg; and 78% TRR and 0.64 mg/kg in seeds. HEPA was low, 1.7% TRR and 0.52 mg/kg in gin trash and 9.6% TRR and 0.08 mg/kg in seeds. No other metabolites exceeded 2% of TRR.

In summary, plant metabolism studies conducted on tomatoes, wheat and cotton indicate that the metabolism of ethephon in these plants was qualitatively similar and indicate that radioactivity penetrated into plants after a foliar application and translocated to edible matrices of plants.

After foliar application to plants, ethephon was metabolized to ethylene and phosphates and HEPA which would be either metabolized to carbon dioxide and phosphate or incorporated into biomolecules such as proteins, carbohydrates and lipids after further metabolism.

In tomatoes, cotton, and wheat hay, most radioactivity was recovered from methanol extracts whilst in wheat grains and straw a significant amount of radioactivity was recovered in the acid hydrolysate, suggesting ethephon is present in conjugated forms.

In tomato and cotton, ethephon was the predominant residue with little HEPA present. However, in wheat grains, HEPA and its conjugates were present at a similar concentration as that of ethephon and its conjugates after the 1× dose and approximately two times higher concentration than ethephon after the 10× exaggerated rate in grain. In wheat hay, HEPA was present at 3.5 times higher than ethephon.

Ethephon would be an appropriate marker for plants except cereal grains and straw in which ethephon was significantly metabolised to HEPA and to conjugates of ethephon and HEPA.

Animal metabolism

The Meeting received information on the fate of orally-dosed [^{14}C]ethephon in lactating goats and laying hens.

Metabolism studies on laboratory animals including rats were reviewed in the framework of toxicological evaluation by the current JMPR.

After oral administration of ethephon to rats, absorption was rapid with a Tmax of 1.0–1.3 hours and 1.9–2.5 hours after a single oral dose of 50 or 1000 mg/kg bw, respectively. Six days after a single dose tissue, and carcass contained only 0.08% or less of administered radioactivity. Highest concentrations were found in liver and kidney. Radioactivity was excreted in urine (47–60%), expired air (18–21%, mainly ethylene) and faeces (4–6.5%), indicating that at least 65% of the administered dose was absorbed. Ethephon was mainly metabolized to ethylene and to a small extent to HEPA.

Two lactating goats were orally administered [^{14}C]ethephon twice daily after am and pm milking in capsules for seven consecutive days at 0.37 and 0.46 mg/kg bw/day (approximately 10 ppm in the diet). The goats were sacrificed approximately 16 hours after the last dose.

A significant portion of the administered dose was released as ethylene (29%) and carbon dioxide (2.0%). Radioactivity was also excreted in urine (19%) and faeces (6.7%). In total, milk contained 3.3% of the administered dose, tissues 3.0%, and content of gastro ontestinatl (GI) tract, 0.84%. Amongst tissues, kidney contained the highest radioactivity at 1.2 mg eq/kg followed by liver at 1.0 mg eq/kg. Fat contained 0.50 mg eq/kg, heart 0.16 mg eq/kg and muscle 0.10 mg eq/kg. Over the study period, average TRR in milk increased from 0.081 mg/kg on day 0.5 to a plateau level of 0.42 mg/kg at day 3.5. The fat fraction of milk contained 45% of the TRR in milk; skimmed milk contained 0.15–0.20 mg eq/kg; and milk fat, 3.0–4.2 mg eq/kg.

In order to estimate ethephon, portions of tissues were hydrolyzed by shaking at 40 °C at pH 11 for one hour to transform ethephon to ethylene. Ethylene released by this hydrolysis was 0.4% TRR in kidney corresponding to 0.008 mg/kg ethephon, 0.05% TRR in fat, and 0% TRR in muscle, liver and milk. Radioactivity in the remaining solids were 0.3%, 2.1%, 71% and 35% of the respective TRR in kidney, liver, muscle and fat.

Extraction of a portion of liver with ether released 5.3% TRR, methanol, a further 64% TRR leaving 27% TRR unextracted. Precipitation with trichloroacetic acid resulted in 12% TRR in liver which is associated with proteins. Glycogen was isolated at a concentration of 0.9 mg/kg.

Two studies were provided on metabolism of ethephon in laying hens. In both studies, hens were orally administered either by capsule or gavage [^{14}C]ethephon at a rate equivalent to 53–67 ppm in the diet for five consecutive days. Hens in the first study were sacrificed 22–23 hours after the last dose and those in the second 9–10 hours after the last treatment.

In the first study, the majority of the administered dose (58%) was recovered as expired ethylene while expired carbon dioxide was negligible. In the excreta, 26–30% of the administered dose was recovered. Liver contained 0.31 mg eq/kg (average), followed by kidney with 0.20 mg eq/kg and fat with 0.15 mg eq/kg. Radioactive residues in the eggs and tissues accounted for less than 1% of the administered dose. Muscle contained 0.023 mg eq/kg showing lower levels than other tissues. Radioactive residues in eggs reached a plateau on Day 4. No identification of metabolites was carried out in this study.

In the second study, approximately one third of the administered dose was recovered in excreta. About 3% of the administered dose was recovered as ethylene but this percentage is not reliable due to the leakage in the experiment. Radioactive residues in the eggs and tissues accounted for less than 1% of the administered dose. Kidneys contained 0.71–1.1 mg eq/kg, liver 0.63–0.90 mg eq/kg, and fat 0.051–0.091 mg eq/kg and muscle, 0.051–0.058 mg eq/kg. Radioactive residues in eggs did not reach a plateau within the study period of 5 days. Higher radioactivity was found in eggs in this study than the first study reaching the level of approximately 0.40 mg eq/kg on Day 5. In eggs, egg yolk contained much higher radioactivity than egg white (1.02 mg eq/kg egg yolk and 0.092 mg eq/kg in egg white).

Ethephon and HEPA were identified in methanol/water extracts of muscle, liver and kidney but not in the hexane/tetrahydrofuran extracts of fat or eggs (both yolk and white). Ethephon was the major residue in kidney accounting for 42% of TRR (0.30 mg/kg) but at a similar level as HEPA in liver (ethephon, 0.11 mg/kg; HEPA, 0.10 mg/kg) and muscle (ethephon, 0.006 mg/kg; HEPA, 0.009 mg/kg). Significant radioactivity was incorporated into amino acids (3–35% of TRR) in these tissues and in fatty acids (around 40% TRR) in fat. Significant amounts of radioactive residues (23 or 40% TRR for liver and 42 or 71% TRR for fat) remain unidentified. In eggs, radioactivity was incorporated into peptides (93% TRR in egg white) and fatty acids/cholesterol/glycerol (77–79% in egg yolk).

In summary, ethephon, when administered orally, was rapidly eliminated either in the excreta or expired as ethylene. Ethephon and HEPA were identified in kidney, liver and muscle in hens. Ethephon was found in kidneys of goats at very low concentrations. Ethephon was metabolized through two routes: metabolized to ethylene and/or to carbon dioxide through HEPA. A similar metabolic pattern was observed in rats, goats and hens. In livestock, radioactivity was found in fatty acids, proteins and glycogen.

Environmental fate

Hydrolysis

Ethephon degrades rapidly at pH 7 and 9 with the half-life of 2.4 and 1.0 day, respectively. At pH 5, it degrades more slowly with a half-life of 73.5 days. Ethylene gas and methylated phosphoric acid were the only degradation products found.

Photochemical degradation

Ethephon showed degradation under continuous irradiation for 360 hours at pH 5 at 25 °C. The half-life was 29 days under irradiation and 51 days without irradiation. Ethephon and ethylene were the only major compounds found. Ethylene was the only degradate of ethephon in the headspace.

Aerobic soil metabolism

The studies on aerobic soil degradation of ethephon in five different soils at 20–25 °C indicate that ethephon applied on soil degraded over time with different rates with the formation of ethylene. DT_{50} values ranged from 2.7–38 days for the five soils tested.

Photolysis on soil surface

Photolysis of ethephon on soil was found to be insignificant. Only ethylene and carbon dioxide were formed.

Field dissipation

Field dissipation studies were conducted at three sites in the USA. In all cases ethephon declined with time. DT_{50} values were 6.8–2 5 days.

Residues in succeeding crops

A confined rotational crop study was conducted to examine the nature and level of residues of ethephon in three succeeding crops (radish, collard and wheat) under outdoor conditions. A single application of radio-labelled ethephon was made on bare plots in plastic containers at a rate of 2.36 kg ai/ha (approximating the highest single application rate for cotton in the USA among approved label rates available to the Meeting). After plant back intervals (PBI) of 30, 120 and 379 days, collard, radish and wheat were planted into the treated soil. Mature radish, collard and wheat were harvested 54–62 days, 68–91 days and 110–158 days after planting. Immature wheat foliage was harvested 47–68 days after planting.

Ethephon declined steadily in soil. Radioactivity in mature plant samples declined in parallel with or faster than the decline in soil. The total extracted radioactive residues were at or lower than 0.07 mg eq/kg in any sample analysed. The solvent extraction recovered 34–37% TRR in 30 day PBI collards, 120 day PBI radish top and 30 day PBI and 120 day PBI wheat forage. As observed in the metabolism study on wheat, only 7.3–24% TRR were extracted by solvents from 30 day PBI and 120 PBI wheat grains and straw.

In the HPLC analysis of plant extracts, where radioactivity was sufficient for characterization, ethephon and HEPA were detected at or below 0.01 mg/kg in the extracts of radish, collard and wheat. No unknown peaks were observed. Sequential treatments of the unextracted radioactive residues for natural components indicated that most of the radioactivity in the plant samples were incorporated into biomolecules, such as starch, proteins, and cellulose fractions.

Overall, ethephon was shown to degrade relatively fast in soil with half-lives around or shorter than the plant back interval of 30 days. The confined succeeding crop study indicated the presence of very low levels of ethephon and HEPA in rotational crops. Therefore, no significant residues of ethephon or HEPA would be expected in rotational crops.

Methods of analysis

Analytical methods for determination of residues of ethephon and its metabolite HEPA were developed for a wide range of matrices of plant and animal origin.

There are three different principles for these analytical methods:

- Ethylene-release by heating in alkaline solution (headspace GC-FID)
- Derivatization to methyl ester using diazomethane (GC-FPD or GC-NPD)
- Extraction: mostly by methanol, acidified methanol or 0.01% formic acid
- LC-MS/MS (m/z 143→ 107 or 145-> 107 and HEPA 125→ 95)
- Extraction: mostly by a mixture of methanol, water and formic acid. Clean-up: mostly with SPE column.

The LC-MS/MS methods were used in the more recent studies.

The methods for plant matrices were validated for ethephon resulting in acceptable mean recoveries and relative standard deviations (RSDs) with the LOQ of 0.01–0.05 mg/kg. They are suitable for determining ethephon in a free form (some methods also for free HEPA).

An LC-MS/MS method was recently developed to determine ethephon and HEPA in both free and conjugated forms in cereal grains, straw and green materials. For the extraction of these compounds, grains and straw were extracted first with methanol and then by a mixture of concentrated hydrochloric acid and water at 50 °C overnight and the extract and hydrolysate were

combined for analysis. For green materials, this acid hydrolysis step was not included. This method was validated for ethephon and HEPA in these matrices resulting in acceptable mean recoveries and RSDs with the LOQ of 0.01 mg/kg for grains and 0.05 mg/kg for straw and green materials.

Methods for animal matrices were validated for ethephon resulting in acceptable mean recoveries and RSDs. The LOQ was 0.002–0.01 mg/kg. They are suitable for determining ethephon in a free form.

A multi-residue method DFG S19 (two variants) was examined for analysis of ethephon in plants for enforcement. However, due to low extraction (30%), this method does not seem appropriate for analysis of ethephon.

Stability of pesticide residues in stored analytical samples

The stability of ethephon was investigated in homogenates of various plant and animal matrices at –20–15 °C at fortification levels 0.2–1.0 mg/kg (plant matrices) or 0.1 mg/kg (animal matrices).

Ethephon was stable when stored frozen for at least 24 months in apples, cherries, grapes, blackberries, pineapples (fruit and forage), melons (36 months), peppers, tomatoes, wheat (grain and straw) and cotton seed (25 months). It was also stable for at least 12 months in apple juice and cotton seed oil.

Ethephon was stable when stored frozen for at least 4 months, the longest period tested, in bovine milk, bovine meat and egg.

Definition of the residue

Plant metabolism studies indicate that ethephon is metabolised in a qualitatively similar pattern in plants. Ethephon penetrates into plants after foliar application and residues of ethephon were found in edible commodities. Ethephon was metabolized to ethylene, which is naturally occurring in plants (but at levels not relevant to MRL setting). Ethephon was metabolized to form HEPA and further metabolized to be incorporated in many biomolecules, such as proteins, carbohydrates and lipids.

In the plants studied, ethephon was the major residue. Except for cereal grains, hay and straw, HEPA was found at much lower concentrations than the parent. In wheat plant fractions, HEPA was present at similar concentrations or higher concentrations than those of ethephon in grain and in hay.

In wheat grains and straw, radioactive residues were recovered at a significant proportion from acid hydrolysate and most of these radioactivity was attributed to ethephon and HEPA. This indicates that ethephon and HEPA were also present in these commodities in the form of conjugates.

The current Meeting considered that HEPA is not a toxicologically relevant metabolite as it does not inhibit cholinesterase activity and the NOAEL for HEPA in a 28-day gavage study in animals is at least two orders of magnitude higher than the NOAEL in humans that formed the basis of the ADI and ARfD.

Residues of ethephon were not expected to occur in significant concentrations in rotational crops.

In summary, the Meeting noted that in cereal grains and straw, presence of ethephon in the form of conjugates is significant. In other plant commodities, the Meeting considered that ethephon would be a good marker for enforcement and for estimation of dietary intake.

One recently developed and validated method, involving methanol extraction and acid hydrolysis/extraction of post methanol-extraction solids is capable of determining total ethephon in free and conjugated forms in cereal matrices. There are other validated methods suitable for determining ethephon in its free form in plant matrices.

In animal metabolism studies, ethephon was rapidly eliminated either in the excreta or exhaled as ethylene. Ethephon was found at low levels in tissues. No metabolites were significant. The Meeting considered that ethephon is a suitable marker for enforcement and for estimation of dietary intake.

There are validated methods available for the determination of ethephon in its free form in animal matrices.

The log K_{ow} (–1.8 to –0.6 at 20 °C) indicates that ethephon is highly water-soluble. Although radioactive residues were found at higher levels in milk fat and egg yolk than skimmed milk or egg white, they were attributed to radioactivity incorporated into fatty acids. The Meeting concluded that the residue is not fat-soluble.

Based on the above, the Meeting recommended the following residue definitions for plant and animal commodities.

Definition of the residue for plant commodities except cereal grains and straw (for compliance with the MRL and for estimation of dietary intake): *Ethephon.*

Definition of the residue for cereal grains and straw (for compliance with the MRL and for estimation of dietary intake*): Ethephon and its conjugates, expressed as ethephon.*

Definition of the residue for animal commodities (for compliance with the MRL and for estimation of dietary intake): *Ethephon.*

The residue is not fat-soluble.

Results of supervised residue trials on crops

The Meeting received supervised trial data for ethephon on apples, cherries, grapes, figs, olives, pineapples, tomatoes (outdoor and indoor), barley, rye, wheat and cotton using foliar sprays of mostly SL formulations containing various concentrations of ethephon.

As ethephon is reviewed under the periodic review programme, the Meeting decided to withdraw its previous recommendations for blueberries, cantaloupes, peppers, dried chilli peppers, hazelnuts and walnuts due to the lack of data.

Apple

A total of 18 supervised trials were conducted on apples in Europe in 2000, 2002, 2006 and 2007, eight in France, two in Germany, one in the UK, two in Italy, two in Spain, one in Portugal and two in Greece.

Residues of ethephon from 13 trials matching critical GAP for apple in France (0.036 kg ai/hL, one to two applications, and pHI 10 days) were: < 0.05, 0.06, 0.07, 0.08, 0.08, 0.14, 0.15, 0.15, 0.24, 0.26, 0.27, 0.40 and 0.49 mg/kg.

The trials matching GAP in France were appropriate for estimating a maximum residue level. The Meeting estimated a maximum residue level of 0.8 mg/kg for apples to replace the previous recommendation. The Meeting also estimated an STMR of 0.15 mg/kg and an HR of 0.49 mg/kg.

Cherries

A total of 15 supervised trials were conducted on cherries in Europe in 2000, 2002 and 2009, ten in France, one in Italy, one in Spain, one in Greece, one in Belgium and one in the Netherlands.

Residues of ethephon from 13 trials matching GAP in Austria for cherries and in the Netherlands for sour cherries (0.36 kg ai/ha, one application, PHI 7 days) were: 0.28, 0.30, 0.33, 0.37, 0.44, 0.52, 0.65, 0.67, 0.91, 1.4, 2.0, 2.3 and 2.7 mg/kg.

The Meeting estimated a maximum residue level of 5 mg/kg for cherries to replace the previous recommendation and an STMR of 0.65 mg/kg and an HR of 2.7 mg/kg.

Grapes

A total of ten supervised trials were conducted on grapes in France in 1995, 2006 and 2009. The GAP in France for grapes allows one application at a maximum rate of 0.45 kg ai/ha with a PHI of 28 days.

Residues from ten trials matching GAP in France were: 0.05, 0.07, 0.14, 0.18, 0.18, 0.20, 0.21, 0.25, 0.37 and 0.52 mg/kg.

The Meeting estimated a maximum residue level of 0.8 mg/kg for grapes to replace the previous recommendation, an STMR of 0.19 mg/kg and an HR of 0.52 mg/kg.

Fig

Six supervised trials were conducted on figs in Brazil in 2004–2005. GAP in Brazil for figs allows one application of 0.94 kg ai/hL with a PHI of 5 days. Ethephon should be applied directly to fruits using brushes with sponge tips or other equipment for even distribution.

Residues from three trials matching GAP in Brazil were, 0.71, 0.73 and 0.75 mg/kg. The Meeting estimated a maximum residue level of 3 mg/kg, an STMR of 0.73 mg/kg and an HR of 0.75 mg/kg for fig.

Olives

Eight supervised trials were conducted on olives in Spain in 2007–2008. GAP in Italy allows two applications (1st application 18 days before harvest at a rate of 0.45 kg ai/ha and 2nd application 11 days before harvest at 0.60 kg ai/ha) with a PHI of 11 days.

Residues from eight trials matching GAP in Italy were, 0.85, 0.90, 0.98, 1.6, 2.2, 2.5, 2.6 and 4.3 mg/kg.

The Meeting estimated a maximum residue level of 7 mg/kg, an STMR of 1.9 mg/kg and an HR of 4.3 mg/kg for olives.

Pineapple

A total of 15 supervised trials were conducted. Five in Brazil in 1994, 1995 and 2005, two in Costa Rica in 1998, two in Côte d'Ivoire in 1997 and 1998, and six in the USA in 1989.

GAP in Kenya for pineapple allows one application at the maximum rate of 1.92 kg ai/ha with a PHI of 7 days. Residues from trial conducted in Côte d'Ivoire matching this GAP were (n=2): 0.11 and 0.97 mg/kg.

Residues from five trials in Brazil matching GAP in Brazil for pineapple (one application at a maximum rate of 0.94 kg ai/ha with a PHI of 14 days) were: < 0.05, 0.11, 0.15, 0.19 and 0.20 mg/kg.

The trials conducted in the USA involved two applications of ethephon and the rate of the first application was two times higher than GAP in Costa Rica (up to two applications at the maximum rate of 1.2 kg ai/ha with a PHI of 1 day; first one 5–7 months before harvest and second 1–2 weeks before harvest) but it was made six months earlier than the expected harvest time with little impact on the residues at harvest.

In the trial in Costa Rica, pineapple was harvested on 0 DALA but as the decline trials indicated that there was no significant decline from 0 to 1 DALA, the Meeting agreed to use the data from 0 DALA.

Residues from trials conducted in the USA and Costa Rica matching GAP in Costa Rica were (n=4), 0.19, 0.22, 0.42 and 0.72 mg/kg. One trial conducted in Brazil matched GAP in Costa Rica and residues were (n=1), 0.47 mg/kg. Combined residue dataset was (n=5), 0.19, 0.22, 0.42, 0.47 and 0.72 mg/kg.

As the dataset from five trials matching GAP in Costa Rica would lead to a higher maximum residue level than the dataset from five trials matching GAP in Brazil, the Meeting decided to use the dataset associated with GAP in Costa Rica. The Meeting estimated a maximum residue level of 1.5 mg/kg to replace its previous recommendation.

The Meeting calculated a mean pulp/whole fruit ratio to be 0.29 using residue levels higher than LOQ. Using the mean and highest residue in whole fruit and this ratio, the Meeting estimated an STMR of 0.12 mg/kg and an HR of 0.21 mg/kg for pineapple.

Tomato

A total of 33 supervised trials on tomatoes were conducted. Twenty-one trials were in Europe in 1999, 2000, 2001 and 2004 and 15 in the USA in 1989–1991 and 2005. As the labels provided to the Meeting do not specify outdoor or indoor uses, the Meeting considered both trials conducted outdoor and indoor.

The critical GAP for the European trials was GAP in Italy which allows the maximum rate of 1.92 kg ai/ha which can be divided into two applications with a PHI of 7 days. Residues from 12 outdoor trials in Europe matching GAP in Italy were 0.24, 0.30, 0.40, 0.45, 0.46, 0.5, 0.55, 0.57, 0.62, 0.68, 0.78, and 0.78 mg/kg.

Residues from nine indoor trials matching GAP in Italy were 0.31, 0.36, 0.45, 0.51, 0.52, 0.66, 0.68, 0.69 and 0.79 mg/kg.

Residues from five independent outdoor trials in the USA matching GAP in Canada (one application of 1.54 kg ai/ha, PHI 14–21 days) were 0.05, 0.06, 0.09, 0.67 and 0.69 mg/kg.

As the outdoor and indoor trials conducted in Europe were in compliance with the same GAP of Italy and they were not significantly different according to Mann-Whitney U test, they could be combined to estimate a maximum residue level. Residues in the combined data set were 0.24, 0.30, 0.31, 0.36, 0.40, 0.45, 0.45, 0.46, 0.5, 0.51, 0.52, 0.55, 0.57, 0.62, 0.66, 0.68, 0.68, 0.69, 0.78, 0.78 and 0.79 mg/kg.

The Meeting confirmed the pervious recommendation of 2 mg/kg for tomato and estimated an STMR of 0.52 mg/kg and an HR of 0.79 mg/kg.

Cereal grains

As the residue definition for cereal grains was recommended to be "ethephon and its conjugates, expressed as ethephon", the Meeting used only those trial data obtained with the recently developed analytical method involving acid hydrolysis to convert ethephon conjugates to free ethephon.

Barley

A total of 53 trials were conducted in Europe in 2000, 2001, 2004, 2007, 2008, 2013 and 2014 on barley.

There are several different groups of GAP in Europe. Critical GAP is either GAP in the UK allowing a maximum single rate of 0.48 kg ai/ha, maximum total rate of 0.48 kg ai/ha, and application timing up to BBCH 49, or GAP in Germany allowing one application at a maximum rate of 0.46 kg ai/ha up to BBCH 49.

Residues from seven trials matching GAP in the UK or Germany were 0.03, 0.07, 0.09, 0.13, 0.23, 0.41, 0.73 mg/kg.

The Meeting estimated, using the dataset matching GAP in the UK or Germany, a maximum residue level of 1.5 mg/kg for barley grains to replace the previous recommendation, and an STMR of 0.13 mg/kg.

Rye

Nine supervised trials were conducted in 2006–2007 in Europe. No data were available on the sum of free and conjugated ethephon in rye grains. (See "Wheat" section below.)

Wheat

A total of 43 supervised trials were conducted on wheat in Europe in 2000, 2001, 2004, 2006, 2007, 2013 and 2014.

There are several different groups of GAP in Europe. Critical GAP is that in Austria and Germany allowing one application at a maximum rate of 0.46 kg ai/ha with application timing up to BBCH 51.

Residues from eight supervised trials matching these GAP were 0.05, 0.06, 0.06, 0.08, 0.11, 0.14, 0.23 and 0.31 mg/kg.

The Meeting estimated, using the dataset from trials matching GAP in Austria and Germany, a maximum residue level of 0.5 mg/kg for wheat grains to replace the previous recommendation, and an STMR of 0.095 mg/kg.

As there are similar GAPs existing for wheat, rye and triticale in countries in Europe, the Meeting decided to extrapolate the maximum residue level and STMR for wheat to rye and triticale.

Cotton seed

A total of ten trials were conducted in Europe in 1993, 1994, 1995 and 2008 on cotton, 41 trials in the USA in 1989, 1993 and 1994, and seven trials in Brazil in 1996 and 2006.

Residues from ten trials conducted in Europe matching GAP in Greece for cotton (one application at a maximum rate of 1.44 kg ai/ha with a PHI of 7 days) were 0.07, < 0.10, < 0.10, < 0.10, 0.10, 0.19, 0.30, 0.35, 0.59 and 1.13 mg/kg.

Residues from six independent trials conducted in Brazil matching GAP in Brazil for cotton (one application at a maximum rate of 1.2 kg ai/ha with a PHI of 7 days) were all below the LOQ: < 0.10 (4) and < 0.20 (2) mg/kg.

Residues from 30 trials matching GAP in the USA for cotton (one application at a maximum rate of 2.24 kg ai/ha with a PHI of 7 days) were 0.06, 0.09, 0.10, 0.11, 0.16, 0.18, 0.23, 0.24, 0.26, 0.26, 0.34, 0.35, 0.36, 0.41, 0.54, 0.55, 0.59, 0.61, 0.65, 0.69, 0.75, 0.86, 1.18, 1.42, 1.50, 2.40, 2.42, 2.73, 2.88 and 4.93 mg/kg.

As the residues from US trials would lead to a higher maximum residue level, the Meeting used the results of the US trials to estimate a maximum residue level. The Meeting estimated a maximum residue level of 6 mg/kg for cotton seed to replace the previous recommendation, and an STMR of 0.545 mg/kg.

Animal feed

Cereal forage

As there is no restriction on feed uses of treated cereal plants, the Meeting used residues in forage samples collected on 0 DALA for cereal forage. Since the determination of ethephon in green materials do not require acid hydrolysis, the Meeting used all available data on barley green material.

Barley forage

Residues in forage collected on 0 DAT from 19 trials matching GAP in the UK or GAP in Germany (a maximum single rate of 0.48 kg ai/ha, maximum total rate of 0.48 kg ai/ha, and application timing up to BBCH 49, or one application at a maximum rate of 0.46 kg ai/ha up to BBCH 49) were 2.6, 3.0, 3.2, 4.2, 4.8, 5.1, 5.7, 6.2, 6.2, 6.2, 6.6, 6.6, 7.7, 7.9, 8.1, 8.4, 9.4, 10 and 11 mg/kg.

Residues from 15 trials matching GAP in France (one application at a maximum application rate of 0.48 kg ai/ha and application timing up to BBCH 39 with a PHI of 56 days) in forage were 3.3, 3.5, 4.2, 4.6, 5.2, 5,6, 5.6, 5.9, 6.0, 6.2, 6.7, 8.1, 8.2, 8.3 and 9.5 mg/kg.

Residues from five trials matching GAP in Poland (one application at a maximum application rate of 0.72 kg ai/ha and application timing up to BBCH 39 were 6.0, 7.1, 8.9, 9.6 and 13 mg/kg.

Residues from seven trials matching another GAP in France (one application at a maximum rate of 0.23 kg ai/ha and application timing up to BBCH 39) were 3.0, 3.7, 4.1, 4.5, 5.2, 5.4, 5.9 and 7.5 mg/kg.

Residues arising from five trials using the application rate of 0.72 kg ai/ha showed higher median and highest residues. Based on this dataset, the Meeting estimated a median residue of 8.9 mg/kg and a highest residue of 13 mg/kg ("as received" basis) for barley forage for animal dietary burden calculation.

Rye forage

Residues in forage collected on 0 DAT from nine trials matching GAP in Germany and Austria (one application at a max rate of 0.73 kg ai/ha, application timing up to BBCH 49) were 4.4, 6.4, 7.2, 7.7, 9.1, 9.2, 9.4, 9.6 and 13 mg/kg.

The Meeting estimated a median and highest residue of 9.1 mg/kg and 13 mg/kg for rye forage on an "as received" basis.

Wheat forage

Residues in forage collected 0 DAT from 17 trials matching GAP in Austria and Germany (one application at a maximum rate of 0.46 kg ai/ha, application timing up to BBCH 51) were 3.1, 3.3, 3.5, 4.0, 4.9, 5.2, 5.9, 6.2, 6.4, 6.5, 7.0, 7.0, 7.1, 7.2, 7.5, 10 and 16 mg/kg.

Residues from 18 trials matching GAP in France (one application at a maximum rate of 0.48 kg ai/ha and application timing up to BBCH 39) were 3.1, 4.5, 4.9, 5.6, 5.7, 6.0, 6.1, 6.9, 7.0, 7.2, 7.4, 7.7, 8.3, 12, 14, 14, 17 and 18 mg/kg

Using the dataset from trials matching GAP in France, the Meeting estimated a median residue of 7.1 mg/kg and a highest residue of 18 mg/kg for wheat forage ("as received" basis).

Cereal straw and fodder, dry

As the residue definition for cereal straw was recommended to be "ethephon and its conjugates, expressed as ethephon", the Meeting used only those trial data obtained using the recently developed analytical method involving acid hydrolysis to convert ethephon conjugates to free ethephon.

Barley straw and fodder, dry

Residues from seven trials matching GAP in the UK or Germany (a maximum single rate of 0.48 kg ai/ha, maximum total rate of 0.48 kg ai/ha, and application timing up to BBCH 49, or one application at a maximum rate of 0.46 kg ai/ha up to BBCH 49) in straw were 0.35, 0.43, 0.51, 0.64, 1.2, 1.5 and 3.6 mg/kg.

Using the data set from the trials matching GAP in the UK or Germany, the Meeting estimated a maximum residue level of 7 mg/kg on a dry weight basis (moisture content of 89%) to replace the previous recommendation. For the purpose of calculation of animal dietary burden, the Meeting estimated a median residue and highest residue of 0.64 mg/kg and 3.6 mg/kg ("as received" basis).

Rye straw and fodder, dry

No data were available on the sum of free and conjugated ethephon in rye straw. (See "Summary of cereal straw and fodder, dry" section below.)

Wheat straw and fodder

Residues from eight trials matching GAP in Austria and Germany (one application at a maximum rate of 0.46 kg ai/ha and application timing up to BBCH 51) in straw were 0.36, 0.44, 0.57, 0.66, 1.2, 1.2, 1.3 and 1.5 mg/kg.

Residues from eight trials matching GAP in France (one application at a maximum rate of 0.48 kg ai/ha and application timing up to BBCH 39 with a PHI of 70 days) in straw were 0.21, 0.29, 0.30, 0.44, 0.84, 0.86, 1.2 and 1.7 mg/kg.

Using the data set from the trials matching GAP in France, the Meeting estimated a median residue of 0.64 mg/kg and a highest residue of 1.7 mg/kg ("as received" basis).

Summary

The Meeting noted that it is not always possible to distinguish straw and fodder of barley, rye, triticale and wheat moving in trade, due to their similarity in appearance. It also noted that there are common or similar GAPs existing for wheat, rye and triticale in countries in Europe. The Meeting decided to extend the maximum residue level recommended for barley straw and fodder at 7 mg/kg on a dry weight basis to straw and fodder of wheat, rye and triticale. The new maximum residue levels for rye and wheat straw and fodder, dry replaces the respective previous recommendations.

The median residue and highest residue estimated for wheat straw and fodder should also apply to rye and triticale straw and fodder, dry.

Cotton gin trash

In 12 US trials, residues in cotton gin trash were analysed and reported. Residues in cotton gin trash from ten trials matching GAP in the USA were: 8.41, 11.1, 13.5, 17.1, 25.1, 28.9, 40.5, 45.5, 54.2 and 55.7 mg/kg. The Meeting estimated a median residue of 27 mg/kg. From the highest residue concentration of individual samples, the Meeting estimated a highest residue of 67 mg/kg.

Fate of residues during processing

High temperature hydrolysis

To simulate the degradation of ethephon during pasteurization, baking, brewing, boiling and sterilisation, the hydrolysis of radio-labelled ethephon was investigated in sterile buffered aqueous solutions.

After incubation at 90 °C (pH 4) for 20 minutes, about 80% of ethephon remained and about 10% was recovered as ethylene. The majority of ethephon was converted to ethylene (76–78%) after incubation at 100 °C (pH 5) for 60 minutes or 120 °C (pH 6) for 20 minutes. Only a minor amount of HEPA was formed.

Processing

The Meeting received information on processing of apple, grapes, olives, tomato, barley, wheat, and cotton seed.

Processing factors calculated for the processed commodities of the above raw agricultural commodities are shown in the table below. STMR-Ps were calculated for processed commodities of apples, grapes, tomatoes, barley, wheat and cotton seed for which maximum residue levels were estimated. Where residues concentrate in processed commodities the Meeting estimated maximum residues levels for these processed commodities using the maximum residue levels for the respective raw agricultural commodities and processing factors.

As no data were available on the processing of fig to dried or dried and candied figs, the Meeting withdrew its previous recommendation on figs, dried and dried and candied.

The processing factor of grape to dried grapes was estimated at 1.2 and therefore a maximum residue level for dried grapes was unnecessary. The Meeting decided to withdraw its previous recommendation on dried grapes.

RAC or Processed commodities	Processing factor		STMR-P	Maximum residue level
	Individual value	Best estimate		
Apple			0.15 (STMR)	0.8
Apple juice	< 0.4, 0.4, 0.5, < 0.8, 1.5	0.5	0.075	–
Apple sauce	0.4, 0.5, < 0.8, 1.1	0.5	0.075	–
Grape			0.19(STMR)	0.8
Dried grapes	0.79, 0.89, 1.0, 1.4, 3.2, 8.5	1.2	0.23	–
Grape juice	0.5, 0.7, 0.8, 1.1	0.75	0.14	–
Must	0.7, 0.8, 0.8, 0.9, 1.0, 1.0	0.85	0.16	–
Wine	0.7, 1.0, 1.2, 1.4, 1.5, 2.1,	1.3	0.25	–
Olives			1.9	–
Olive oil (virgin and refined)	< 0.02, < 0.03	< 0.02	0.038	–
Table olives	< 0.01, < 0.02, < 0.02, < 0.03	< 0.01	0.019	–
Tomato			0.52(STMR)	2
Tomato juice	< 0.1, 0.1, < 0.2, 0.34	0.22	0.18	–
Tomato puree	< 0.1, < 0.1, < 0.2, 0.60	0.60	0.31	–
Tomato paste	0.5, 0.6, 0.75	0.6	0.31	–
Tomato preserves	< 0.1, < 0.2, 0.2	0.2	0.10	–
Barley			0.13(STMR)	1.5
Pearl barley	0.9	0.9	0.12	–
Wheat			0.095 (STMR)	0.5
Flour	0.1, 0.2, < 0.3,	0.15	0.014	–
Wheat germ	2.0	2.0	0.19	1
Wheat bran	1.4, 3.1, 3.5	3.1	0.29	1.5
Cotton seed			0.545 (STMR)	6
Cottonseed refined oil	< 0.02,< 0.03, < 0.03	< 0.02	0.011	–

For the purpose of calculating animal dietary burden, the Meeting estimated the following median residues for feed items.

RAC or Processed commodities	Processing factor		median residue
	Individual value	Best estimate	
Apple			0.15 (STMR)
Wet pomace	0.3, 0.4, 0.6, < 0.8, 1.1	0.5	0.075
Dry pomace	2.0	2.0	0.30
Grape			0.19(STMR)
Wet pomace	0.4, 0.6, 0.9, 1.1	0.75	0.14
Tomato			0.52(STMR)
Wet pomace	< 0.1, < 0.1, < 0.2, 0.52	0.52	0.27
Dry pomace	1.9	1.9	0.99
Barley			0.13(STMR)
Barley hulls	1.6	1.6	0.21
Cotton seed			0.55 (STMR)
Meal	0.02, 0.03, 0.07	0.03	0.016

Residues in animal products

Farm animal feeding studies

Lactating cows received oral administration of ethephon at dose rates equivalent to 44, 128 and 415 ppm in the diet once daily for 28 consecutive days. The residues of ethephon in whole milk appeared to reach plateau after Day 4. Ethephon in milk was 0.007 mg/kg at 44 ppm dose, 0.02 mg/kg at 128 ppm dose, and 0.03 mg/kg at the 415 ppm dose. After a 28 day-administration, the highest concentration of ethephon in kidney was 0.58, 3.2 and 7.8 mg/kg respectively after 44, 128 and

415 ppm dose. In liver, it was 0.08, 0.51 and 0.99 mg/kg. In muscle, the ethephon concentration was much lower at 0.01, 0.05 and 0.12 mg/kg for these dose groups. In fat, at the highest dose, ethephon was present at only 0.06 mg/kg.

Laying hens were orally administered with ethephon at rates equivalent to 2.3, 6.9 and 23 ppm in the diet once daily for 28 consecutive days. The residues of ethephon in whole eggs were very low and those from the highest dose group contained at a maximum 0.0036 mg/kg. Therefore, eggs from the 2.3 ppm and 6.9 diets were not analyzed. After 28-day administration, liver contained the highest concentration of ethephon, 0.033 mg/kg at the 2.3 ppm dose, 0.068 at the 6.9 ppm dose and 0.29 ppm at the 23 ppm dose. In skin + fat, it was 0.014, 0.032 and 0.117 mg/kg. In muscle, it was 0.060 at 23 ppm diet.

Estimation of dietary burdens

The maximum and mean dietary burdens were calculated using the highest and median residues of ethephon estimated at the current Meeting on a basis of the OECD Animal Feeding Table. In Australia, use of ethephon-treated cereal green materials as feed is not allowed and cereal forage is not in trade. Residues arising from use of ethephon in barley, rye and wheat forages were not used for calculating animal dietary burden for the Australian diets.

Summary of livestock dietary burdens (ppm of dry matter diet)

	US-Canada		EU		Australia		Japan	
	Max	Mean	max	Mean	Max	Mean	Max	mean
Beef cattle	4.19	1.65	18.8	9.14	4.04	0.81	0.13	0.13
Dairy cattle	14.5	6.22	18.9 [a]	9.17 [b]	1.46	0.79	0.059	0.059
Broilers	0.11	0.11	0.10	0.10	0.024	0.024	0.015	0.015
Layers	0.11	0.11	7.33 [c]	3.17 [d]	0.024	0.024	0.012	0.012

[a] Suitable for estimating maximum residue levels for milk, meat, fat and edible offal of cattle

[b] Suitable for estimating STMRs for milk, meat, fat and edible offal of cattle

[c] Suitable for estimating maximum residue levels for eggs, meat, fat and edible offal of poultry

[d] Suitable for estimating STMRs for eggs, meat, fat and edible offal of poultry

Residues in milk and cattle tissues

The maximum and mean dietary burdens in cattle were 18.9 and 9.17 ppm of dry matter diet respectively for estimating a maximum residue level and STMR for milk and edible tissues. The maximum residue levels, STMRs and HRs for relevant commodities of mammal origin were estimated using the residue levels in tissues and milk at 0 and 44 ppm feeding groups.

	Feed level (ppm) for milk residues	Ethephon (mg/kg) in milk	Feed level (ppm) for tissue residues	Ethephon (mg/kg) in Muscle	Liver	Kidney	Fat
Maximum residue level beef or dairy cattle							
Feeding study [a]	0 44	– 0.002	0 44	< 0.01 0.016	0.05 0.095	0.03 0.64	< 0.01 < 0.01
Dietary burden and highest residue	18.9	0.0009	18.8	0.007	0.069	0.29	0.004
STMR beef or dairy cattle							
Feeding study [b]	0 44	– 0.002	0 44	< 0.01 0.01	0.05 0.08	0.03 0.49	< 0.01 < 0.01
Dietary burden and mean residue	9.17	0.0004	8.25	0.002	0.056	0.13	0.002

[a] Highest residues for tissues and mean residue for milk

[b] Mean residues for tissues and mean residue for milk

The level < LOQ at 0 ppm dose is assumed to be 0 mg/kg residue.

The Meeting estimated STMRs of 0.0004, 0.002, 0.056, 0.13 and 0.002 mg/kg, and HRs of 0.0009, 0.007, 0.069, 0.29 and 0.004 mg/kg for milk, meat, liver and kidney respectively.

On a basis of highest residues above, the Meeting estimated maximum residue levels of 0.01 *, 0.01 *, 0.4 and 0.01 * mg/kg for milks mammalian meat, edible offal and fat, respectively.

The previous recommendations for milk of cattle, goats and sheep, meat of cattle, goats, houses, pigs and sheep, and edible offal of cattle, goats, horses, pigs and sheep were withdrawn.

Residues in eggs and chicken tissues

The maximum and mean dietary burdens in poultry were 7.33 and 3.17 ppm of dry matter diet respectively for estimating a maximum residue level and STMR for eggs and edible tissues. The maximum residue levels, STMRs and HRs for relevant commodities of poultry origin were estimated using the residue levels in tissues and eggs at 2.3, 6.9 and 23 ppm feeding groups.

	Feed level (ppm) for egg residues	Ethephon (mg/kg) in			
		Eggs	Muscle	Liver	Fat [a]
Maximum residue level broiler or layer hens					
Feeding study	6.9 23	na 0.0023	0.015 0.060	0.068 0.23	0.032 0.117
Dietary burden and highest residue	7.33	0.00005	0.016	0.072	0.034
STMR broiler or layer hens					
Feeding study	2.3 6.9	na na	< 0.01 0.012	0.031 0.062	0.013 0.024
Dietary burden and mean residue	3.17	0 [b]	0.01	0.037	0.015

[a] From data in fat + skin

[b] At a dose of 23 ppm in the dry matter diet, residues were 0.0036 mg/kg

The Meeting estimated STMR of 0, 0.01, 0.037 and 0.015 mg/kg, and HR of 0.00005, 0.016, 0.072 and 0.034 mg/kg, respectively for poultry eggs, meat, edible offal and fat.

On a basis of HR, the Meeting estimated maximum residue levels of 0.01 *, 0.02, 0.08 and 0.04 mg/kg for eggs, poultry meat, edible offal and fat, respectively. The recommendations for poultry meat and edible offal replace the previous recommendations.

The Meeting withdrew its previous recommendation on chicken eggs.

RECOMMENDATIONS

On the basis of the data from supervised trials the Meeting concluded that the residue levels listed in Annex 1 are suitable for establishing maximum residue limits and for IEDI and IESTI assessment.

Definition of the residue for plant commodities except cereal grains and straw (for compliance with the MRL and for estimation of dietary intake): *Ethephon.*

Definition of the residue for cereal grains and straw (for compliance with the MRL and for estimation of dietary intake): *Ethephon and its conjugates, expressed as ethephon.*

Definition of the residue for animal commodities (for compliance with the MRL and for estimation of dietary intake): *Ethephon.*

The residue is not fat-soluble.

DIETARY RISK ASSESSMENT

Long-term intake

The International Estimated Dietary Intakes (IEDIs) of ethephon were calculated for the 17 GEMS/Food cluster diets using STMRs and STMRPs estimated by the current Meetings (Annex 3). The ADI is 0–0.05 mg/kg bw and the calculated IEDIs were 0–6% of the maximum ADI. The Meeting concluded that the long-term intake of residues of ethephon resulting from the uses considered by the current JMPR is unlikely to present a public health concern.

Short-term intake

The International Estimated Short-Term Intakes (IESTI) of ethephon were calculated for commodities using HRs/HR-Ps and STMRs/STMR-Ps estimated by the current Meeting (see Annex 4). The ARfD is 0.05 mg/kg and the calculated IESTIs were 0–100% of the ARfD for the general population and 0–70% of the ARfD for children. The Meeting concluded that the short-term intake of residues of ethephon, when used in ways that have been considered by the JMPR, is unlikely to present a public health concern.