5.3 ACETOCHLOR (280)

TOXICOLOGY

Acetochlor is the ISO-approved common name for 2-chloro-*N*-(ethoxymethyl)-*N*-(2-ethyl-6-methylphenyl)acetamide (IUPAC), with CAS number 34256-82-1. It belongs to the group of chloroacetanilide compounds, which are used as herbicides. Acetochlor is a pre-emergence herbicide used against grasses and broadleaf weeds in corn, soya beans, sorghum and peanuts grown in high organic content. It inhibits protein synthesis in shoot meristems and root tips.

Acetochlor has not previously been evaluated by JMPR and was reviewed by the present Meeting at the request of CCPR.

All critical studies contained statements of compliance with GLP.

Biochemical aspects

Following gavage dosing of rats, acetochlor was rapidly and almost completely absorbed and rapidly excreted. Male bile duct–cannulated rats given 10 mg/kg bw excreted on average 85.1% of the dose in bile, 8.0% in urine and 4.2% in faeces over 48 hours. Similar results were obtained with the high dose and following repeated oral dosing. No pronounced sex differences were observed following the administration of single low or high doses or repeated dosing. Radiolabel was widely distributed throughout the body (< 4% of the administered dose in tissues and carcass); highest concentrations were observed in blood (but not in plasma), liver, heart, lung and spleen. There was some accumulation in nasal turbinates in rats, but not in mice. The combined urinary and faecal excretion showed biphasic elimination, with a half-life of 5.4–10 hours for the α phase and 162–286 hours for the β phase.

Acetochlor was extensively metabolized. No parent compound was detected in the urine, and less than 1% was found in faeces. Species differences were observed, particularly with respect to the formation of sulfur-containing precursors to the dialkylbenzoquinoneimine (DABQI) metabolites that are believed to be responsible for the induction of nasal tumours in rats. The primary metabolic pathway in the rat involves O-dealkylation and subsequent glucuronidation or glutathione conjugation, enterohepatic circulation and excretion. The predominant metabolite in rat plasma following oral administration of acetochlor was a secondary amide, *S*-methyl sulfoxide. In contrast, in mice, acetochlor is metabolized primarily to a number of glucuronides, which are excreted in the urine. In Rhesus monkeys, glutathione conjugation and subsequent metabolism via the mercapturic acid pathway occur preferentially. However, as a result of the higher molecular weight threshold for biliary excretion in primates compared with rats, the metabolites appear to be excreted primarily via the urine and not the bile and thus would not be subjected to the formation of *S*-methyl sulfoxide or other *S*-methyl metabolites, as in rats.

Toxicological data

In rats, the acute oral LD_{50} was 1929 mg/kg bw, the acute dermal LD_{50} was 4166 mg/kg bw and the acute inhalation LC_{50} was greater than 2.1 mg/L. Acetochlor was severely irritating to the skin of rabbits and mildly irritating to the eyes of rabbits. It was a skin sensitizer in guinea-pigs, as determined by the Buehler test and Magnusson and Kligman maximization test. It gave a positive response for phototoxicity in an in vitro mouse fibroblast assay.

The finding observed most consistently in short- and long-term toxicity studies in mice, rats and dogs is decreased body weight, with changes in haematology and clinical chemistry in some studies.

In a 91-day toxicity study in mice using dietary acetochlor concentrations of 0, 800, 2000 and 6000 parts per million (ppm) (equivalent to 0, 120, 300 and 900 mg/kg bw per day, respectively), the NOAEL was 2000 ppm (equivalent to 300 mg/kg bw per day), based on decreased body weight observed at 6000 ppm (equivalent to 900 mg/kg bw per day).

In a 29-day toxicity study in rats using dietary acetochlor concentrations of 0, 300, 600, 1200, 2400, 4800 and 9600 ppm (equal to 0, 33.3, 67.7, 132, 267, 519 and 1012 mg/kg bw per day for males and 0, 35.2, 69.3, 139, 279, 539 and 1081 mg/kg bw per day for females, respectively), the NOAEL was 600 ppm (equal to 67.7 mg/kg bw per day), based on slight decreases in body weight gain and prothrombin time observed at 1200 ppm (equal to 132 mg/kg bw per day).

Two 3-month dietary toxicity studies were conducted in rats. In the first study, using dietary acetochlor concentrations of 0, 800, 2000 and 6000 ppm (equal to 0, 53.2, 134 and 460 mg/kg bw per day for males and 0, 69.3, 173 and 530 mg/kg bw per day for females, respectively), marginal decreases in feed consumption and body weight gain were observed at 800 ppm (equal to 53.2 mg/kg bw per day), the lowest dose tested.

In the second study, using dietary acetochlor concentrations of 0, 20, 200 and 2000 ppm (equal to 0, 1.60, 16.1 and 161 mg/kg bw per day for males and 0, 1.92, 18.8 and 191 mg/kg bw per day for females, respectively), the NOAEL was 200 ppm (equal to 16.1 mg/kg bw per day), based on significantly decreased feed consumption and body weight gain seen at 2000 ppm (equal to 161 mg/kg bw per day).

The overall NOAEL for the two 3-month toxicity studies in rats was 200 ppm (equal to 16.1 mg/kg bw per day). The overall LOAEL was 800 ppm (equal to 53.2 mg/kg bw per day).

In a 91-day toxicity study in dogs administered acetochlor by capsule at a dose of 0, 2.0, 10.0 or 60.0 mg/kg bw per day, the NOAEL was 10.0 mg/kg bw per day, based on decreased body weight gain, clinical signs, increased relative liver weights, increased serum alanine aminotransferase activity and increased blood glucose levels observed in both sexes and reduced haemoglobin, haematocrit and erythrocyte counts observed in females at 60.0 mg/kg bw per day.

In a 119-day toxicity study in dogs administered acetochlor by capsule at a dose of 0, 25.0, 75.0 or 200 mg/kg bw per day, a NOAEL could not be identified, as effects were observed at all doses. At the LOAEL of 25.0 mg/kg bw per day, decreased red blood cell counts, decreased haematocrit, increased alkaline phosphatase activity and increased relative adrenal and liver weights occurred in females.

Two 1-year toxicity studies in dogs were conducted. In the first study, in which dogs were administered acetochlor by capsule at a dose of 0, 4.0, 12.0 or 40.0 mg/kg bw per day, the NOAEL was 12.0 mg/kg bw per day, based on decreased body weights and feed consumption, testicular atrophy, increased liver and kidney weights and decreased testis weight observed at 40.0 mg/kg bw per day.

In the second study, in which dogs were administered acetochlor by capsule at a dose of 0, 2.0, 10.0 or 50.0 mg/kg bw per day, the NOAEL was 2.0 mg/kg bw per day, based on decreased feed consumption and body weight gain in females and changes in kidneys (interstitial nephritis) and tubular degeneration in testes in males observed at 10.0 mg/kg bw per day.

The overall NOAEL for the two 1-year toxicity studies in dogs was 2.0 mg/kg bw per day. The overall LOAEL was 10.0 mg/kg bw per day.

Two long-term toxicity and carcinogenicity studies were conducted in mice. In the first study, in which mice were given acetochlor in the diet at a concentration of 0, 500, 1500 or 5000 ppm (equal to 0, 75, 227 and 862 mg/kg bw per day for males and 0, 95, 280 and 1084 mg/kg bw per day for females, respectively) for 23 months, increases in absolute and relative kidney weights (both sexes) and liver weights (males only), a dose-related increase in interstitial nephritis in both sexes and retinal degeneration in females (positive trend) were observed at all dose levels. The high dose of 5000 ppm was considered to be excessive and above the maximum tolerated dose (MTD). Statistically significant increases in the incidence of lung tumours in females of all dose groups were considered not to be related to treatment based on the consideration of HCD. The Meeting noted that the incidence of histiocytic sarcomas in females at 1500 ppm was marginally outside the historical control range for the performing laboratory, that this tumour occurs commonly in aged mice and that this tumour is of unknown relevance to humans.

In the second study, in which mice were administered acetochlor in the diet at a concentration of 0, 10, 100 or 1000 ppm (equal to 0, 1.10, 11.0 and 116 mg/kg bw per day for males and 0, 1.40, 13.0 and 135 mg/kg bw per day for females, respectively) for 78 weeks, the NOAEL was 10 ppm (equal to 1.10 mg/kg bw per day), based on slight anaemia and increased incidences of bronchiolar hyperplasia and interstitial fibrosis in the kidney in males observed at 100 ppm (equal to 11.0 mg/kg bw per day). A significantly increased incidence of renal tubular basophilia was also noted in males at 10 and 100 ppm; however, these increased incidences were not considered to be adverse because they were of minimal severity and because of the lack of a clear dose–response relationship, the lack of associated histopathological findings, the lack of similar effects in females or in either sex at higher dose levels in a study of longer duration and the lack of corroborative renal findings. There was a slight increase in the incidence of adenoma in the lungs of males and females at 1000 ppm; however, the increase was not considered to be treatment related based on the lack of a dose–response relationship, low incidence in concurrent controls, the lack of an increase in tumour multiplicity with increasing dose and the comparable incidence of lung tumours in historical controls.

Three long-term toxicity and carcinogenicity studies have been conducted in rats. In a 27-month study using dietary acetochlor concentrations of 0, 500, 1500 and 5000 ppm (equal to 0, 22.0, 69.0 and 250 mg/kg bw per day for males and 0, 30.0, 93.0 and 343 mg/kg bw per day for females, respectively), decreased body weight in males was observed at 500 ppm, the lowest dose tested. Treatment-related neoplastic findings (liver, thyroid and nasal tumours) occurred at 5000 ppm, but this dose was well above the MTD. An increased incidence of nasal tumours was observed at 1500 ppm (equal to 69.0 mg/kg bw per day).

In the second study, in which rats were administered acetochlor in the diet at a concentration of 0, 40, 200 or 1000 ppm (equal to 0, 1.9, 9.4 and 47.5 mg/kg bw per day for males and 0, 2.4, 11.8 and 60.0 mg/kg bw per day for females, respectively) for 24 months, the NOAEL was 200 ppm (equal to 9.4 mg/kg bw per day), based on decreased body weight in males, elevated total bilirubin in females and elevations of gamma-glutamyltransferase activities and cholesterol levels in males at 1000 ppm (equal to 47.5 mg/kg bw per day). An increased incidence of papillary adenomas of the nasal mucosa was observed at 1000 ppm (equal to 47.5 mg/kg bw per day).

In the third study, in which rats were administered dietary acetochlor at a concentration of 0, 18, 175 or 1750 ppm (equal to 0, 0.67, 6.4 and 66.9 mg/kg bw per day for males and 0, 0.88, 8.5 and 92.1 mg/kg bw per day for females, respectively) for 24 months, the NOAEL was 175 ppm (equal to 6.4 mg/kg bw per day), based on reduced body weight and feed consumption, changes in the eyes (degeneration of outer retinal layer), reduced blood cell parameters and increased incidence of focal hyperplasia in the nasal epithelium at 1750 ppm (equal to 66.9 mg/kg bw per day). Adenomas and carcinomas of nasal epithelium and thyroid adenoma were observed at 1750 ppm (equal to 66.9 mg/kg bw per day).

The overall NOAEL for systemic toxicity in the three long-term toxicity studies in rats was 200 ppm (equal to 9.4 mg/kg bw per day), and the LOAEL was 500 ppm (equal to 22.0 mg/kg bw per day).

A comparative 52-week toxicity study was conducted in rats using a dietary acetochlor concentration of 1750 ppm (equal to 99.6 mg/kg bw per day) and a dietary *sec*-amide methylsulfoxide concentration of 100/150/300 ppm (equal to 14.6 mg/kg bw per day). The results of this study clearly demonstrate that the sulfoxide metabolite of acetochlor is a nasal carcinogen in the rat. The development, morphology and location of the tumours were identical to those seen with acetochlor. The tumorigenic potency of the sulfoxide metabolite is also comparable with that of acetochlor.

A number of mode of action studies are available for acetochlor, indicating that the nasal tumours observed in rats likely result from the site- and species-specific formation of reactive quinoneimine metabolites within the nasal epithelium and the associated formation of adducts with nasal proteins. It is postulated that this results in cytotoxicity, based on the observation of inflammation and metaplasia in vivo, leading to prolonged nasal cell proliferation and eventual development of nasal olfactory tumours. Because of large differences in the in vitro rate of formation of quinoneimines in human and rat nasal tissues and in the rate of formation of quinoneimine adducts

in vivo in rats, mice and squirrel monkeys, this mode of action is unlikely to lead to nasal tumours in humans at levels of exposure arising from pesticide residues in food.

Mechanistic studies have shown that the thyroid tumours observed in rats are caused by induction of hepatic uridine diphosphate-glucuronosyltransferase (UDPGT). This, in turn, leads to decreased levels of thyroid hormone and a compensatory increase in thyroid stimulating hormone (TSH), which acts upon the rat thyroid to induce hyperplasia and, ultimately, neoplasia. Therefore, thyroid tumours in rats are not considered relevant to humans because of the well-known differences in thyroid hormone homeostasis between rats and humans.

The Meeting concluded that acetochlor induces tumours in mice of unknown relevance for humans and that the modes of action for the nasal epithelial and thyroid tumours in rats have been established.

Acetochlor has been evaluated in an adequate range of in vitro and in vivo genotoxicity studies. Acetochlor exhibited weak positive responses in some in vitro gene mutation assays conducted with less than pure material. It was clastogenic at cytotoxic concentrations in human lymphocytes; this response is attributable to the metabolism of acetochlor to the chloromethyl group and low glutathione levels. However, no evidence of clastogenicity or other genotoxic effects was observed in a number of in vivo assays, including a rat bone marrow chromosomal aberration assay, two mouse micronucleus assays and several dominant lethal mutation assays in rats and mice. No evidence of DNA damage was noted in an in vitro unscheduled DNA synthesis (UDS) assay in rat hepatocytes. A weakly positive response was noted in an in vivo rat hepatocyte UDS assay, but only at a high dose level (2000 mg/kg bw, which is higher than the LD_{50} value) associated with hepatic glutathione depletion and severe hepatotoxicity.

The Meeting noted the absence of a specific assay for gene mutations in vivo. However, the Meeting concluded that, on the basis of the weight of evidence, acetochlor was unlikely to be genotoxic in vivo.

Acetochlor is unlikely to be genotoxic in vivo, the histiocytic sarcomas in mice are commonly observed as animals age and occur only at high doses and the proposed modes of action for tumours of the thyroid and nasal epithelium in rats involve a threshold and are highly unlikely to occur in humans. Therefore, the Meeting concluded that acetochlor is unlikely to pose a carcinogenic risk to humans from the diet.

Three two-generation reproductive toxicity studies in rats are available. In the first study, in which rats were given diets containing acetochlor at a concentration of 0, 200, 600 or 1750 ppm (equal to 0, 18.6, 57.0 and 160 mg/kg bw per day for F_0 males and 0, 21.5, 64.6 and 199 mg/kg bw per day for F_0 females, respectively), the NOAEL for parental toxicity was 200 ppm (equal to 21.5 mg/kg bw per day), based on decreased body weight in F_1 females at 600 ppm (equal to 64.6 mg/kg bw per day). The NOAEL for offspring toxicity was 200 ppm (equal to 18.6 mg/kg bw per day), based on decreased F_2 litter size at birth, decreased F_1 and F_2 pup body weights during lactation, and decreased absolute and relative spleen weights in F_2 weanlings observed at 600 ppm (equal to 57.0 mg/kg bw per day). The NOAEL for reproductive toxicity was 200 ppm (equal to 18.6 mg/kg bw per day), based on a decreased number of implantations seen at 600 ppm (equal to 57.0 mg/kg bw per day).

In the second study, in which rats were given diets containing acetochlor at a concentration of 0, 18, 175 or 1750 ppm (equal to 0, 1.3, 12.6 and 124 mg/kg bw per day for F_0 males and 0, 1.6, 15.5 and 157 mg/kg bw per day for F_0 females, respectively), the NOAEL for parental toxicity was 175 ppm (equal to 12.6 mg/kg bw per day), based on decreases in body weight, body weight gain and feed consumption and increases in relative weights of brain, kidney, liver and spleen in F_1 females and of testes, seminal vesicles and thymus in F_1 males at 1750 ppm (equal to 124 mg/kg bw per day). The NOAEL for offspring toxicity was 175 ppm (equal to 12.6 mg/kg bw per day), based on decreased pup body weights observed at 1750 ppm (equal to 124 mg/kg bw per day). The NOAEL for reproductive toxicity was 1750 ppm (equal to 124 mg/kg bw per day), the highest dose tested.

In the third study, in which rats were given diets containing acetochlor at a concentration of 0, 500, 1500 or 5000 ppm (equal to 0, 30.8, 90.6 and 316 mg/kg bw per day for F_0 males and 0, 46.2, 130 and 157 mg/kg bw per day for F_0 females), the NOAEL for parental toxicity was 500 ppm (equal to 30.8 mg/kg bw per day), based on decreased body weights and decreased relative liver and kidney weights at 1500 ppm (equal to 90.6 mg/kg bw per day). The NOAEL for offspring toxicity was 1500 ppm (equal to 90.6 mg/kg bw per day), based on decreased body weights of F_{2b} pups at 5000 ppm (equal to 316 mg/kg bw per day). The NOAEL for reproductive toxicity was 5000 ppm (equal to 316 mg/kg bw per day), the highest dose tested.

The overall NOAEL for parental toxicity was 200 ppm (equal to 21.5 mg/kg bw per day), and the overall LOAEL was 600 ppm (equal to 64.6 mg/kg bw per day). The overall NOAELs for reproductive and offspring toxicity were 200 ppm (equal to 18.6 mg/kg bw per day). The overall LOAELs for reproductive and offspring toxicity were 600 ppm (equal to 57.0 mg/kg bw per day).

Two developmental toxicity studies in rats are available. In the first study, which used oral gavage acetochlor doses of 0, 40.0, 150 and 600 mg/kg bw per day, the NOAEL for maternal toxicity was 150 mg/kg bw per day, based on mortality, clinical signs of toxicity, decreased body weight gain and feed consumption and a marked increase in water consumption observed at 600 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 150 mg/kg bw per day, based on a reduction in mean fetal weight and reduced ossification at 600 mg/kg bw per day.

In the second study, which used oral gavage acetochlor doses of 0, 50.0, 200 and 400 mg/kg bw per day, the NOAEL for maternal toxicity was 200 mg/kg bw per day, based on decreased body weight gains and clinical signs of toxicity seen at 400 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 400 mg/kg bw per day, the highest dose tested.

The overall NOAEL and LOAEL for maternal toxicity in rats were 200 and 400 mg/kg bw per day, respectively. The overall NOAEL and LOAEL for embryo and fetal toxicity in rats were 400 and 600 mg/kg bw per day, respectively.

Two developmental toxicity studies in rabbits are available. In the first study, which used oral gavage acetochlor doses of 0, 30.0, 100 and 300 mg/kg bw per day, the NOAEL for maternal toxicity was 100 mg/kg bw per day, based on decreased feed consumption and body weight (GDs 6–8) and the death of two dams at 300 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 300 mg/kg bw per day, the highest dose tested.

In the second study, which used oral gavage acetochlor doses of 0, 15.0, 50.0 and 190 mg/kg bw per day, the NOAEL for maternal toxicity was 50.0 mg/kg bw per day, based on decreased body weight at GD 19 and decreased body weight gain during GDs 7–19 at 190 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 190 mg/kg bw per day, the highest dose tested.

The overall NOAEL and LOAEL for maternal toxicity in rabbits were 100 and 190 mg/kg bw per day, respectively. The overall NOAEL for embryo and fetal toxicity in rabbits was 300 mg/kg bw per day, the highest dose tested.

The Meeting concluded that acetochlor is not teratogenic in rats or rabbits.

In an acute neurotoxicity study in rats administered a single oral gavage acetochlor dose of 0, 150, 500 or 1500 mg/kg bw, decreased body weights and body weight gain and reduced feed consumption were observed at 1500 mg/kg bw. No neurotoxicity was observed.

In a 90-day study of neurotoxicity in rats given diets containing acetochlor at a concentration of 0, 200, 600 or 1750 ppm (equal to 0, 15.4, 47.6 and 139.0 mg/kg bw per day for males and 0, 18.3, 55.9 and 166.5 mg/kg bw per day for females, respectively), marginal decreases in mean body weight and body weight gain in males and females were observed at 1750 ppm (equal to 139.0 mg/kg bw per day). There was no evidence for neurotoxicity or neuropathological effects up to 1750 ppm (equal to 139.0 mg/kg bw per day), the highest dose tested.

The Meeting concluded that acetochlor is not neurotoxic.

No evidence of immunotoxicity was observed in an immunotoxicity study in female mice administered acetochlor in the diet at a dose level of 0, 500, 1500 or 5000 ppm (equal to 0, 119, 334 and 1536 mg/kg bw per day, respectively) for 28 days.

The Meeting concluded that acetochlor is not immunotoxic by the oral route of exposure.

Biochemical and toxicological data on metabolites and/or degradates

Biochemical and toxicological studies were conducted on plant metabolites, soil degradates and environmental metabolites of acetochlor.[1] The absorption, distribution, metabolism and excretion studies in rats and mice indicate that some of these metabolites were absorbed, minimally metabolized and rapidly excreted. There is no significant accumulation in the body. The acute oral LD_{50} values were greater than 2000 mg/kg bw in rats except for two metabolites, for which LD_{50} values were slightly lower than 2000 mg/kg bw in female rats. The 28-day and 90-day dietary toxicity studies in rats indicate NOAELs of 3000 ppm (equal to 225 mg/kg bw per day) and above, primarily based on decreases in body weight. No evidence of thyroid toxicity or effects on the nasal epithelium was observed in these studies. No evidence of embryo/fetal toxicity was observed at 1000 mg/kg bw per day in rats. No evidence of genotoxicity was observed in various in vivo and in vitro assays, except for a mouse lymphoma assay, which gave a weak positive response for two metabolites; however, these two metabolites were negative in a mouse micronucleus assay.

The Meeting concluded that these plant metabolites, soil degradates and environmental metabolites of acetochlor appear to be less toxic than the parent compound.

Human data

There were no reports of adverse health effects in manufacturing plant personnel. Also, there were no reports of poisonings with acetochlor. A recent epidemiological study reported weak associations between exposure to acetochlor and a number of human cancers. However, the authors stated that a lack of exposure–response trends, the small number of exposed cases and the relatively short time between acetochlor use and cancer development prohibit definitive conclusions.

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

Toxicological evaluation

The Meeting established an ADI of 0–0.01 mg/kg bw on the basis of a NOAEL of 1.10 mg/kg bw per day in the 78-week dietary study in mice, based on slight anaemia and an increased incidence of bronchiolar hyperplasia and interstitial fibrosis in the kidney in males observed at 11.0 mg/kg bw per day. A safety factor of 100 was applied.

The upper bound of the ADI provides a margin of exposure of at least 28 000 relative to the LOAEL for histiocytic sarcomas in mice (280 mg/kg bw per day) and 4700 relative to the LOAEL for nasal tumours in rats (47.5 mg/kg bw per day).

An ARfD of 1 mg/kg bw was established on the basis of a NOAEL of 100 mg/kg bw per day in a study of developmental toxicity in rabbits, based on decreased feed consumption, decreased body weight (GDs 6–8) and the death of two dams observed at 300 mg/kg bw per day. A safety factor of 100 was applied.

A toxicological monograph was prepared.

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[1] *N*-(6-ethyl-3-hydroxy-2-methylphenyl) oxamic acid (68), acetochlor *t*-ethanesulfonic acid (7), acetochlor *t*-oxanilic acid (2), acetochlor *t*-sulfinylacetic acid (3), acetochlor *s*-ethanesulfonic acid (13), *t*-norchloroacetochlor (6) and *t*-hydroxyacetochlor (17). The numbers in parentheses refer to the EU reference number.

Levels relevant to risk assessment of acetochlor

Species	Study	Effect	NOAEL	LOAEL
Mouse	Twenty-three-month study of toxicity and carcinogenicity[a]	Toxicity	–	500 ppm, equal to 75 mg/kg bw per day[b]
		Carcinogenicity	500 ppm, equal to 95 mg/kg bw per day	1 500 ppm, equal to 280 mg/kg bw per day
	Seventy-eight-week study of toxicity and carcinogenicity[a]	Toxicity	10 ppm, equal to 1.10 mg/kg bw per day	100 ppm, equal to 11.0 mg/kg bw per day
		Carcinogenicity	1 000 ppm, equal to 116 mg/kg bw per day[c]	–
Rat	Two-year studies of toxicity and carcinogenicity[a,d]	Toxicity	200 ppm, equal to 9.4 mg/kg bw per day	500 ppm, equal to 22.0 mg/kg bw per day
		Carcinogenicity	500 ppm, equal to 22.0 mg/kg bw per day	1 000 ppm, equal to 47.5 mg/kg bw per day
	Two-generation studies of reproductive toxicity[a,d]	Reproductive toxicity	200 ppm, equal to 18.6 mg/kg bw per day	600 ppm, equal to 57.0 mg/kg bw per day
		Parental toxicity	200 ppm, equal to 21.5 mg/kg bw per day	600 ppm, equal to 64.6 mg/kg bw per day
		Offspring toxicity	200 ppm, equal to 18.6 mg/kg bw per day	600 ppm, equal to 57.0 mg/kg bw per day
	Developmental toxicity studies[d,e]	Maternal toxicity	200 mg/kg bw per day	400 mg/kg bw per day
		Embryo and fetal toxicity	400 mg/kg bw per day	600 mg/kg bw per day
	Acute neurotoxicity study[e]	Neurotoxicity	1 500 mg/kg bw[c]	–
	Ninety-day neurotoxicity study[a]	Neurotoxicity	1 750 ppm, equal to 139 mg/kg bw per day[c]	–
Rabbit	Developmental toxicity study[d,e]	Maternal toxicity	100 mg/kg bw per day	190 mg/kg bw per day
		Embryo and fetal toxicity	300 mg/kg bw per day[c]	–
Dog	Ninety-day and 1-year studies of toxicity[d,e]	Toxicity	2 mg/kg bw per day	10 mg/kg bw per day

[a] Dietary administration.
[b] Lowest dose tested.
[c] Highest dose tested.
[d] Two or more studies combined.
[e] Gavage administration, including capsules.

Estimate of acceptable daily intake (ADI)

0–0.01 mg/kg bw

Estimate of acute reference dose (ARfD)

1 mg/kg bw

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

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

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

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapidly and extensively absorbed from gastrointestinal tract (≥ 93% in 48 h)

Dermal absorption	Low; 9.7% in Rhesus monkey
Distribution	Widely distributed; highest concentrations in blood (but not plasma), liver, heart, lung, spleen and nasal turbinates
Potential for accumulation	No evidence of accumulation
Rate and extent of excretion	Rapid; ~77% excreted within first 24 h following a single low dose
Metabolism in animals	Extensive, species differences in metabolism, *sec*-amide mercapturic acid in rats and glucuronides in mice
Toxicologically significant compounds in animals and plants	Acetochlor
Acute toxicity	
Rat, LD_{50}, oral	1 929 mg/kg bw
Rabbit, LD_{50}, dermal	4 166 mg/kg bw
Rat, LC_{50}, inhalation	> 2.1 mg/L (4 h)
Rabbit, dermal irritation	Severely irritating
Rabbit, ocular irritation	Mildly irritating
Guinea-pig, dermal sensitization	Sensitizing (maximization test and Buehler test)
Short-term studies of toxicity	
Target/critical effect	Kidney and testes
Lowest relevant oral NOAEL	2.0 mg/kg bw per day (dog)
Lowest relevant dermal NOAEL	400 mg/kg bw per day (highest dose tested; rabbit)
Lowest relevant inhalation NOAEC	No data
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Anaemia, kidney and liver (mouse, rat)
Lowest relevant oral NOAEL	1.10 mg/kg bw per day (mouse)
Carcinogenicity	Adenomas in nasal epithelium (rat)[a]
Genotoxicity	
	Unlikely to be genotoxic in vivo[a]
Reproductive toxicity	
Target/critical effect	Decreased body weights in adults and pups, liver and kidney weights in adults, decreased number of implantations per dam
Lowest relevant parental NOAEL	21.5 mg/kg bw per day (rat)
Lowest relevant offspring NOAEL	18.6 mg/kg bw per day (rat)
Lowest relevant reproductive NOAEL	18.6 mg/kg bw per day (rat)
Developmental toxicity	
Target/critical effect	Decreased body weights and mortality (rat, rabbit), decreased fetal weight and reduced ossification (rat)
Lowest relevant maternal NOAEL	100 mg/kg bw per day (rabbit)
Lowest relevant embryo/fetal NOAEL	300 mg/kg bw per day (rabbit)
Neurotoxicity	
Acute neurotoxicity NOAEL	1 500 mg/kg bw per day (highest dose tested; rat)

Subchronic neurotoxicity NOAEL	139 mg/kg bw per day (highest dose tested; rat)
Developmental neurotoxicity NOAEL	No data
Other toxicological studies	
Immunotoxicity NOAEL	1 536 mg/kg bw per day (highest dose tested; mouse)
Mechanistic studies	Nasal tumours: Studies establishing a rat-specific mode of action involving a production of cytotoxic quinoneimine metabolites that result in compensatory hyperplasia leading to tumours Thyroid tumours: Studies establishing a rodent-specific mode of action for thyroid tumours
Medical data	
	No adverse effects reported in workers at manufacturing plants and agricultural workers

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

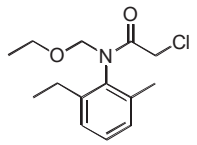
Summary

	Value	Study	Safety factor
ADI	0–0.01 mg/kg bw	Seventy-eight-week toxicity study (mouse)	100
ARfD	1 mg/kg bw	Developmental toxicity study (rabbit)	100

RESIDUE AND ANALYTICAL ASPECTS

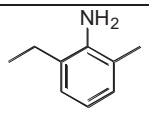
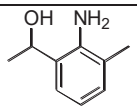
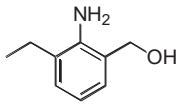
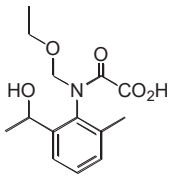
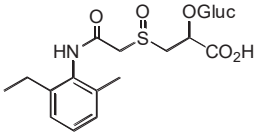
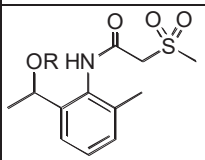
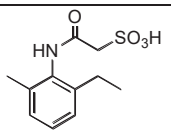
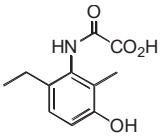
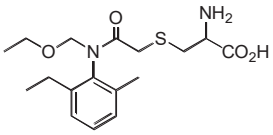
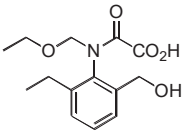
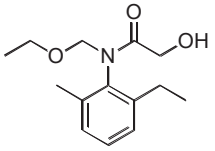
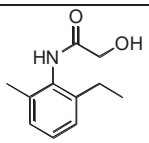
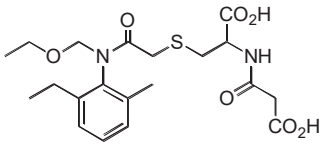
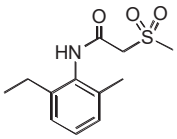
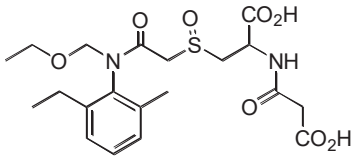
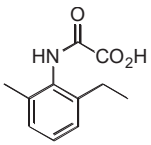
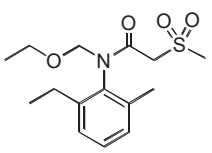
Acetochlor is a selective herbicide which, after application, is absorbed mainly by the shoots of germinating plants, and to some extent, by roots. Acetochlor is used as a pre-emergence or early post-emergence soil-applied herbicide. Acetochlor controls annual grasses and broadleaf weeds, germinating from seeds; however, its action against perennial weeds is very limited. At the Forty-sixth Session of the CCPR (2014), it was scheduled for the evaluation as a new compound by 2015 JMPR.

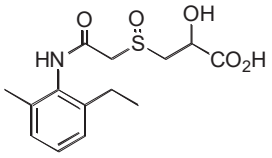
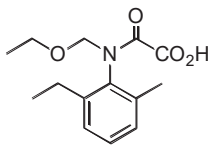
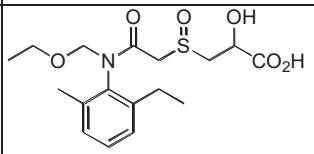
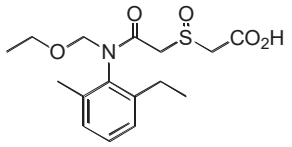
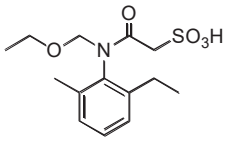
The Meeting received information on the metabolism of acetochlor in maize, soya beans and cotton, lactating goats and cows, laying hens, follow crops, methods of residue analysis, freezer storage stability, GAP information, supervised residue trials on maize (forage, grain, stover and silage), sweet corn (forage, kernels plus cob with husks removed, stover and silage), cotton (gin by-products and seed), sorghum (grain, forage and stover), soya bean (meal and seed), sugar beet (dried pulp, roots, tops, sugar and molasses), peanuts (hay and meal) and livestock transfer studies (lactating cows and laying hens).



Acetochlor is 2-chloro-*N*-(ethoxymethyl)-*N*-(2-ethyl-6-methylphenyl)acetamide

Metabolites referred to in the appraisal were addressed by their common names with the corresponding aniline metabolite class (EMA, HEMA, HMEA or OH) indicated in brackets.

2-ethyl-6-methyl aniline = EMA		2-(1-hydroxyethyl)-6-methyl aniline = HEMA	
2-ethyl-6-hydroxymethyl aniline = HMEA			
1-hydroxyethyl *tert*-oxanilic acid (HEMA class)		*sec*-sulfinyllactic acid glucose conjugate (EMA class)	
1-hydroxyethyl-*sec*-methylsulfone glucosylsulfate conjugate (HEMA class)	 R=glucose sulfate	*sec*-sulfonic acid (EMA class)	
5-hydroxy-*sec*-oxanilic acid (OH class)		*tert*-cysteine (EMA class)	
hydroxymethyl-*tert*-oxanilic acid (HMEA class)		*tert*-hydroxyacetochlor (EMA class)	
sec-hydroxyacetochlor (EMA class)		*tert*-malonylcysteine (EMA class)	
sec-methylsulfone (EMA class)		*tert*-malonylcysteine sulfoxide (EMA class)	
sec-oxanilic acid (EMA class)		*tert*-methylsulfone (EMA class)	

sec-sulfinyllactic acid (EMA class)		*tert*-oxanilic acid (EMA class)	
tert-sulfinyllactic acid (EMA class)		*tert*-sulfinylacetic acid (EMA class)	
tert-sulfonic acid (EMA class)			

Plant metabolism

Acetochlor is typically used for three different situations:

- Incorporation into the soil prior to planting the crop (PP)
- As a broadcast spray to weeds and bare soil after seeding but prior to crop emergence (PE)
- As a broadcast spray to weeds and the growing crop, i.e. post-emergence (PO).

The Meeting received plant metabolism studies with acetochlor following pre-plant, pre- and post-emergent applications to maize (corn), cotton and soya bean.

Maize

The metabolism of [^{14}C-U-phenyl]-acetochlor in maize grown outdoors was studied following either a pre-emergence (PE) application immediately after seeding or post-emergence after allowing the corn plants to grow to a height of 66–71 cm (growth stage V6 to V7, i.e., 6–7 leaves fully emerged) before spraying. The effective treatment rates were 3.6 kg ai/ha for the PE application and 3.5 kg ai/ha for the PO application.

Total radioactive residues in PE forage, grain and stover were 0.67, 0.04 and 1.84 mg equiv/kg while those in PO forage, grain and stover were higher at 3.44, 0.022 and 6.41 mg equiv/kg respectively.

Solvent (CH_3CN/H_2O) extracted ≥ 79% of the TRR present in immature plants, forage and stover samples. Extraction of ^{14}C present in grain was lower at 58–63% TRR. The majority of the ^{14}C present in the solids after extraction were associated with natural products, especially starch, protein, lignin and hemicellulose. A large number of metabolites were detected in the solvent extracts but not unchanged acetochlor. There were notable differences in the pattern of metabolites observed following PE compared to PO application.

The metabolites identified in PO forage and stover primarily resulted from initial glutathione conjugation of acetochlor followed by oxidation to give sulfoxide-type metabolites. Only one compound exceeded 10% of TRR: *tert*-sulfinyllactic acid was observed at 12.6% TRR (0.43 mg equiv/kg) in forage and 11.3% of TRR (0.72 mg equiv/kg) in stover. Two other metabolites exceeded 0.1 mg equiv/kg: *sec*-sulfinyllactic acid and *sec*-sulfinyl lactic acid glucose conjugate.

In contrast, in PE maize the compounds detected resulted largely from the uptake of soil metabolites to give oxanilate-type metabolites. None of the individual components exceeded 10% of TRR in immature plant, forage or stover. The major component was 5-hydroxy *sec*-oxanilic acid

present at levels of 8.4% (0.099 mg equiv/kg) 6.2% (0.042 mg equiv/kg) and 4.3% (0.080 mg equiv/kg) TRR in immature plants, forage and stover respectively.

In grain from PE or PO application, no individual compound exceeded 10% of TRR and no discrete component characterized by chromatography exceeded 0.001 mg equiv/kg.

Compounds containing an intact phenyl ring can be classified according to the aniline that would be generated on base hydrolysis. Non-hydroxylated metabolites give EMA, those hydroxylated at the 1-position of the ethyl side-chain give HEMA, those at the hydroxylated at the methyl side-chain HMEA and those hydroxylated at the 3, 4 or 5 positions of the phenyl ring could be classed as "OH" anilines. The major aniline metabolite class observed in maize (PE and PO) is EMA followed by OH.

Soya bean

The metabolism of [^{14}C-U-phenyl]-acetochlor in soya beans grown outdoors following either a pre-plant (PP) or post-emergence (PO) application was studied. The PP application was made to the soil (loamy sand) 45 days before seed planting while the PO application was made to a second group of plants 42 days after planting seed when the plants were approximately at the R1–R2 growth stage (beginning flowering to full flowering). The application rates were 3.5 kg ai/ha for the PP and 3.7 kg ai/ha for the PO application.

Levels of radioactivity were higher in PO treated plants compared to PP application. TRRs were 1.67 and 11.4 mg equiv/kg in PP and PO forage, respectively; 3.48 and 57.7 mg equiv/kg in PP and PO hay; and 0.175 and 0.192 mg equiv/kg in PP and PO seed.

Solvent (CH_3CN/H_2O) extracted ≥ 86% of the TRR present in forage and hay samples. Extraction of ^{14}C present in grain was lower at 59–80% TRR.

As was the case with maize, a large number of metabolites were detected in the solvent extracts but not unchanged acetochlor. There were also notable differences in the patterns of metabolites observed following PP compared to PO application.

Like maize, the metabolites identified in PO soya bean forage and hay primarily resulted from initial glutathione conjugation of acetochlor followed by oxidation to give sulfoxide-type metabolites. Five compounds exceeded 10% of TRR: *tert*-cysteine (forage 39% TRR, 4.45 mg equiv/kg), *tert*-malonylcysteine (forage and hay 18–23%TRR, 2.62–10.6 mg equiv/kg), *tert*-sulfinyllactic acid and *tert*-malonylcysteine sulfoxide (forage and hay; combined 24–30%TRR, 2.72–17.3 mg equiv/kg). A large number of other metabolites were present at levels in excess of 0.1 mg equiv/kg.

In contrast, in PP soya bean forage or hay the compounds detected resulted largely from the uptake of soil metabolites to give oxanilate-type metabolites. None of the individual components exceeded 10% of TRR in immature plant, forage or hay. The major metabolites were *tert*-oxanilic acid (> 9.5% TRR, > 0.158 mg equiv/kg) in forage and *tert*-oxanilic acid combined with *tert*-sulfonic acid present at levels of > 9.7% (0.34 mg equiv/kg) in hay.

Both PP and PO seed extracts contained numerous low-level metabolites (≥ 27), none of which exceeded 0.03 mg equiv/kg. PP seed metabolites were generally more polar than PO seed metabolites.

The major aniline metabolite classes in soya bean commodities are EMA and "other" for PP forage, HEMA and EMA for PP hay and EMA for PO hay.

Cotton

The metabolic fate of [^{14}C-U-phenyl]-acetochlor in cotton maintained outdoors was examined following either a pre-plant (PP) soil (sandy loam) application 30 days before seed planting or as a separate application (PO) made to plants 15 days after the majority of plants had reached their first white flower stage. The application rates were 3.6 kg ai/ha for the PP and for the PO application.

TRR in PO leaves/stems were 63.9 mg equiv/kg whilst the TRR in PP leaves/stems were much lower at 5.7 mg equiv/kg. The TRRs in seed from both treatments were similar at 0.13 mg equiv/kg for the PO treatment and 0.10 mg equiv/kg for the PP treatment.

Solvent (CH_3CN/H_2O) extracted ≥ 88% of the TRR present in leaf/stem samples. Extraction of ^{14}C present in seed was lower at 29–44% TRR.

In contrast to maize and soya bean, the metabolites identified following PP and PO applications were both from initial conjugation of acetochlor with glutathione, followed by subsequent loss of glutamate, then glycine. The resulting cysteinyl product underwent oxidation, deamination, dealkylation, and further conjugation with malonate or glucose to produce numerous metabolites. Only one compound exceeded 10% of TRR in PP leaves/stems: 1 hydroxyethyl-*sec*-methylsulfone glucosylsulfate conjugate (> 15%TRR, > 0.85 mg equiv/kg) and one following PO application: *sec*-sulfinyllactic acid (20% TRR, 12.5 mg equiv/kg). Levels of ^{14}C in cotton seed were too low to allow identification of the numerous metabolites present, none of which individually exceeded 5.3% TRR or 0.007 mg equiv/kg.

The major aniline metabolite classes in cotton leaves and stems are EMA and HEMA.

In summary, the metabolism of acetochlor by plants is well understood. Primary metabolic pathways of acetochlor in plants included:

1) hydrolytic/oxidative dechlorination to form the alcohol (and conjugates) and subsequent oxidation of the alcohol to the oxanilic acid

2) displacement of chlorine by glutathione (or homoglutathione) and further catabolism of the products to cysteine or lactic acid metabolites, and the S-oxides and conjugates, or to sulfonic acids and methyl sulfones

3) ethyl/methyl side-chain or ring hydroxylation; and 4) N dealkylation. Oxanilate, sulfonic acid, and sulfone metabolites were more prevalent in PP and PE matrices. Glutathione/homoglutathione conjugation followed by catabolism to cysteine and lactic acid metabolites, and their oxidized derivatives and conjugates, was the primary metabolic pathway for acetochlor after PO treatment.

Animal metabolism

The plant metabolism studies show that livestock are unlikely to be exposed to parent acetochlor. Rather, animals will be exposed to a range of metabolites, none of which is considered likely to be a major component of the residue. A range of livestock metabolism studies were made available to the meeting including the metabolism of acetochlor in lactating goats and laying hens as well as the metabolism of a range of plant metabolites administered individually or as a combination to lactating animals (goats and cows) or laying hens.

Acetochlor

Lactating goats were orally dosed twice daily for four consecutive days with [^{14}C-U-phenyl]-acetochlor at a dose equivalent to 8.1 to 11 ppm in the feed. The majority of the ^{14}C residues was recovered in the excreta (urine 58–71%AD, faeces 20–29% AD). For tissues, ^{14}C residues were highest in liver, (0.277–0.588 mg equiv/kg), followed by the kidney (0.247–0.479 mg equiv/kg), muscle TRR ranged from (0.012 to 0.024 mg equiv/kg) and fat (0.002–0.003 mg equiv/kg). TRR in milk reached 0.016 mg equiv/kg after two days of dosing. No intact acetochlor was detected in tissues or milk. The majority of the residues were not recovered by mild extraction techniques using organic solvents or water at ambient temperatures. Cell fractionation confirmed the ^{14}C in the solids had been incorporated into natural products, principally proteins.

Laying hens were orally dosed once a day for seven consecutive days with [^{14}C-U-phenyl]-acetochlor at a dose equivalent to 10 ppm in the feed. The majority of the ^{14}C residues was recovered in the excreta (68–72.3%AD). Radioactivity reached its highest level in eggs on Day 7 from the start of dosing, with average concentrations of 0.072 mg equiv/kg for yolk and 0.007 mg equiv/kg for egg

whites. Mean levels of TRR were 0.337 mg equiv/kg in liver, 0.054 mg equiv/kg in breast muscle, 0.072 mg equiv/kg in leg muscle, 0.019 mg equiv/kg in peritoneal fat, and 0.041 mg equiv/kg in skin plus subcutaneous fat. No intact acetochlor was detected in tissues or eggs. The majority of the residue was associated with natural products; proteins, glycan, and lipid fractions.

Metabolism of selected acetochlor plant metabolites by livestock

1-hydroxyethyl-tert-sulfonic acid

Groups of lactating goats were dosed orally with ^{14}C-[1-hydroxyethyl-*tert*-sulfonic acid] for five or 28 consecutive days at a dose equivalent to 0.4 to 5.7 ppm in the feed. In an animal dosed at the equivalent of 5.7 ppm for five days, most of the ^{14}C was recovered in the excreta (faeces 68.7%AD, and urine 3.65% AD). TRR in tissues was very low, with 0.007 mg equiv/kg (1-hydroxyethyl-*tert*-sulfonic acid equivalents) in kidney, 0.003 mg equiv/kg in liver, and < 0.0003 mg equiv/kg in muscle and fat. For animals dosed for 28 days, ^{14}C residues in milk and tissues were < 0.001 mg equiv/kg.

Metabolism of four acetochlor plant metabolites co-administered to lactating goat

Two lactating goats were orally dosed with a mixture of metabolites (*tert*-sulfonic acid, *tert*-oxanilic acid, *tert*-hydroxyacetochlor and *tert*-sulfinylacetic acid ratio 25:19:13:1 based on weight) uniformly labelled in the phenyl ring at 13.7 mg acetochlor equivalents/goat twice daily for five days equivalent to 3.2 and 4.3 ppm (acetochlor equivalents) in the feed. Most of the ^{14}C was excreted (63–79% AD) with similar amounts recovered in urine (34–42% AD) and faeces (29–37% AD). Residues in milk reached a plateau by the fourth day of dosing. Levels of ^{14}C were highest in kidney (0.034 mg acetochlor equiv/kg) followed by liver (0.022 mg equiv/kg) with levels in muscle and fat below the limit of detection. Levels of ^{14}C in milk were 0.006 mg equiv/kg. The HPLC profile of urine and faeces was similar to the dosing solution suggesting limited transformation occurs. Due to the low levels of ^{14}C present in tissue, analysis was by high pressure acid hydrolysis to form anilines. The only aniline metabolite class observed was EMA, the same as the dosing compounds.

Laying hens were dosed with the same mixture of metabolites (but in ratios 1:1:1:1 based on weight) for five to six days at doses equivalent to 13 to 88 ppm (acetochlor equivalents) in the feed. Excreta and cage wash accounted for ≥ 96% AD. The highest levels of ^{14}C found in the tissues of the hens dosed with 88 ppm were in liver (0.150–0.266 mg equiv/kg) followed by kidneys (0.106–0.128 mg equiv/kg) with much lower levels found in fat (0.049–0.061 mg equiv/kg) and muscle (0.024–0.032 mg equiv/kg). Egg whites and yolks collected at sacrifice had ^{14}C residue levels that ranged from 0.029 to 0.052 mg equiv/kg and from 0.192 to 0.198 mg equiv/kg, respectively.

The main components of ^{14}C detected in tissues and eggs were unchanged *tert*-hydroxyacetochlor (2.9–26%TRR) and *tert*-oxanilic acid 1.2–20.4% TRR) as well as *sec*-oxanilic acid (6.3% TRR yolk).

Metabolism of 5-hydroxy-sec-oxanilic acid in lactating cow

A metabolite of acetochlor in maize, 5-hydroxy-*sec*-oxanilic acid, uniformly labelled in the phenyl ring was used to dose a lactating cow at a nominal rate of 25 ppm (30 ppm if expressed in acetochlor equivalents) in the diet for seven consecutive days. Most of the administered dose was recovered from the excreta (faeces 82.5% and urine 8.4%).

The residues in all tissues and milk were < 0.01 mg 5-hydroxy-sec-oxanilic acid equiv/kg, except in the kidney which had a residue of 0.015 mg equiv/kg. Extraction of ^{14}C residues in kidney with CH_3CN:H_2O released 70% of the TRR. In kidney unchanged 5-hydroxy-*sec*-oxanilic acid accounted for 46.7%TRR with the remainder composed of unextracted material (24.5%TRR) and uncharacterized aqueous soluble residues (15.0%TRR).

The metabolism of acetochlor and selected plant metabolites (*tert*-oxanilic acid, *tert*-sulfonic acid, *tert*-sulfinylacetic acid, *sec*-sulfonic acid, *tert*-norchloroacteochlor, 5-hydroxy-*sec*-oxanilic acid?) in laboratory animals (rats) was summarized and evaluated by the WHO panel of the JMPR in the present meeting.

In summary, the metabolism of acetochlor in goats is similar to metabolism in laboratory animals. Studies on a limited number of plant metabolites suggests, at least for these plant metabolites, that following oral dosing they remain the major component of the ^{14}C residues.

Environmental fate

The Meeting received information on soil aerobic metabolism, aqueous photolysis and aqueous hydrolysis properties of [^{14}C]acetochlor. Studies were also received on the behaviour of [^{14}C]acetochlor in a rotational crop situation.

The degradation of acetochlor in soil maintained under aerobic conditions is rapid with four major degradates identified; *tert*-oxanilic acid, *tert*-hydroxy, *tert*-sulfonic acid and *tert*-sulfinylacetic acid. While parent acetochlor is degraded relatively quickly in soils the degradates formed are moderately persistent. In the laboratory studies, soil DT_{50} values for parent acetochlor ranged from 3.3 to 55 days while for field dissipation studies DT_{50} values ranged from 2.9 to 12.6 days.

Acetochlor was stable to hydrolysis in aqueous solutions at pH 5, 7 and 9 (25 °C) suggesting hydrolysis plays a negligible role in its degradation. Similarly negligible degradation was observed in an aqueous photolysis study suggesting photolysis is not a major route of degradation.

In a confined rotational crop study with lettuce, radish and wheat, a plot of sandy loam soil was treated with [^{14}C-U-phenyl]-acetochlor at the equivalent of 2.24 or 3.36 kg ai/ha and crops sown 30, 120 and 365 days after the soil application. Analysis of soil extracts prior to planting showed that acetochlor was degraded to an array of compounds, many of which were present at very low levels. In addition to acetochlor, four major soil degradates were identified as present in soil throughout the study: *tert*-oxanilic acid, *tert*-sulfonic acid, *tert*-sulfinylacetic acid and *tert*-hydroxyacetochlor.

Five compounds, which were consistently present in plant extracts from all three rotation intervals were: *sec*-oxanilic acid (0–11%TRR; < LOD–0.075 mg equiv/kg; not observed in grain), *tert*-oxanilic acid (0–25%TRR; < LOD–0.17 mg equiv/kg; up to 0.003 mg equiv/kg in grain), *sec*-sulfonic acid (0–27%TRR; < LOD–0.21 mg equiv/kg; not observed in grain), *tert*-sulfonic acid (0–16%TRR; < LOD–0.072 mg equiv/kg; up to 0.0045 mg equiv/kg in grain), and 1-hydroxyethyl *tert*-oxanilic acid (0–15%TRR; < LOD–0.43 mg equiv/kg; up to 0.0017 mg equiv/kg in grain). Unextracted radioactive residues in plant matrices were characterized by cell wall fractionation. The majority of this plant bound material was incorporated into hemicellulose and cellulose and in the case of wheat grain into starch.

The major aniline metabolite class in rotational crop types was EMA except in wheat grain for which it was HMEA and HEMA.

In a separate study [^{14}C-U-phenyl]-acetochlor was applied to the surface of a sandy loam soil at a nominal rate equivalent to 3.08 kg ai/ha. Mustard, turnip and millet were planted approximately 30, 120 and 365 days after [^{14}C]acetochlor application. Soya beans were planted approximately 30 and 365 days after treatment. The radioactive residues dissipated rapidly in soil with only 22% AR remaining 30 days after application. The main identified soil degradates were *tert*-oxanilic acid, *tert*-sulfinylacetic acid and *tert*-sulfonic acid.

Analyses of the plant extracts showed that extensive metabolism occurred in all crops. Acetochlor was not found in any of the RACs analysed, With the exception of Day 30 turnip roots, acetochlor was not found in RACs. The ^{14}C residue levels decreased in crops from the 30 day compared to the 365 days planting. The TRR was partially characterized and found to be comprised of up to nine different compounds, with not one above 0.01 mg equiv/kg in the edible portion of the root or cereal crop (turnip root and millet grain). The major metabolites identified in crops planted 30 DAA were *tert*-oxanilic acid, *sec*-methyl sulfone, *sec*-hydroxyacetochlor, and *tert*-methyl sulfone.

The major aniline class of metabolites was EMA in which no hydroxylation of the alkyl groups of the phenyl ring had occurred with HEMA class metabolites was also significant.

In summary, acetochlor related residues in soil may contribute to residues observed in rotational and primary crops.

Methods of Analysis

The metabolism of acetochlor in crops results in a complex mixture of metabolites, most of which produce EMA or HEMA on base hydrolysis. Any non-metabolised parent acetochlor that might be present would be converted to EMA upon hydrolysis.

Consequently most of the methods developed to quantify acetochlor residues in animal and plant commodities involve hydrolytic conversion of metabolites to the EMA and HEMA chemophores. These analytes are quantified and expressed in acetochlor equivalents and then may be added to give total acetochlor residues. LOQs are typically 0.01 mg/kg each for EMA and HEMA.

The methods all involve initial extraction of samples with an organic/aqueous solvent mixture, typically CH_3CN/H_2O, followed by hydrolysis of residues with aqueous hydroxide solutions. The main differences between methods involve clean-up conditions and instrumentation for quantification, LC-MS/MS in more recent versions.

Representative compounds that generate EMA (*tert*-sulfonic acid) and HEMA (1-hydroxyethyl-*tert*-oxanilic acid) on base hydrolysis are used as reference materials for fortification and method validation.

The methods are suitable for analysis of acetochlor and related metabolites in plant and animal matrices.

Multi-residue methods are currently not validated for acetochlor and its metabolites.

Stability of pesticide residues in stored analytical samples

The Meeting received information on the stability of acetochlor and example metabolites hydrolysable to EMA (*tert*-sulfonic acid) and HEMA (1-hydroxyethyl-*tert*-oxanilic acid) and for some matrices HMEA (hydroxymethyl-*tert*-oxanilic acid) and OH-class (5-hydroxy-*sec*-oxanilic acid) in various matrices on freezer storage (–18 °C).

Residues of parent acetochlor were stable in potato tubers for at least 295 days and sugar beet tops for at least 294 days storage.

Residues of *tert*-sulfonic acid (EMA-class) and 1-hydroxyethyl-*tert*-oxanilic acid (HEMA class) measured using a common moiety method, were stable in alfalfa forage and clover hay for at least 330 days freezer storage, soya bean forage for 390 days, soya bean hay for 391 days, soya bean grain for 382 days, wheat forage for 741 days, wheat straw for 741 days, wheat grain for 734 days, sorghum silage for 739 days, sorghum grain for 732 days, potato tubers for 286 days, sugar beet tops for 286 days, maize grain for 356 days, maize forage for 357 days and maize stover for 351 days.

Residues of hydroxymethyl-*tert*-oxanilic acid (HMEA class) measured using a common moiety method, were stable in sorghum grain, silage for at least 732 days, soya bean grain, forage and hay for at least 380 days and wheat grain, forage and straw for at least 734 days.

Residues of 5-hydroxy-*sec*-oxanilic acid (OH-class) measured using a common moiety method, were stable in maize grain, forage and stover, lettuce, turnip roots and leaves and soya bean seed and hay for at least 730 days.

Residues of a mixture of *tert*-hydroxyacetochlor, *tert*-oxanilic acid, *tert*-sulfonic acid and *tert*-sulfinylacetic acid (EMA-class) in equal proportions measured using a common moiety method, were stable in eggs, milk, chicken liver, pig liver, beef liver, muscle, fat, and kidneys for at least 910 days.

The periods of demonstrated stability cover the frozen storage intervals used in the residue studies.

Definition of the residue

Following application of acetochlor to crops (maize, soya bean and cotton) a large number of metabolites were detected, but not unchanged acetochlor. There were notable differences in the

pattern of metabolites observed following applications (PP and PE) to soil prior to crop emergence compared to applications made when the crop is present (PO).

The metabolites identified in forage, stover and hay following PO application to maize and soya beans are mainly sulfoxide-type metabolites. Significant metabolites (> 10%TRR) were *tert*-sulfinyllactic acid (13% TRR 0.43 mg equiv/kg maize forage; 11% TRR 0.72 mg equiv/kg maize stover), *tert*-cysteine (39% TRR soya hay), *tert*-malonylcysteine (18–23%TRR soya bean forage and hay), *tert*-sulfinyllactic acid and *tert*-malonylcysteine sulfoxide (combined 24–30%TRR soya bean forage and hay).

In contrast, in PE maize and PP soya beans the compounds detected resulted largely from the uptake of soil metabolites to give oxanilate-type metabolites. None of the individual components exceeded 10% of TRR in immature plant, forage, stover or hay. The major metabolite in PE maize was 5-hydroxy *sec*-oxanilic acid present at levels of 8.4% (0.099 mg equiv/kg) 6.2% (0.042 mg equiv/kg) and 4.3% (0.080 mg equiv/kg) TRR in immature plants, forage and stover respectively. The major metabolites in soya bean were *tert*-oxanilic acid (> 9.5% TRR) in forage and combined with *tert*-sulfonic acid present at levels of > 9.7% (0.34 mg equiv/kg) in hay.

Metabolism of acetochlor in cotton differed compared to maize and soya bean in that the metabolites identified following both PP and PO applications were from initial conjugation of acetochlor with glutathione, followed by subsequent loss of glutamate, then glycine. Only one compound exceeded 10% of TRR in PP leaves/stems: 1 hydroxyethyl-*sec*-methylsulfone glucosylsulfate conjugate (14.8%TRR) and one following PO application: *sec*-sulfinyllactic acid (20% TRR).

Negligible residues were detected in seeds and grain. Metabolites detected in maize were individually present at < 0.001 mg/kg. Identification of individual metabolites was not achieved in soya bean grain and cotton seed. In both cases extracts contained numerous metabolites, each present at < 0.03 mg equiv/kg (soya bean grain) or < 0.01 mg equiv/kg (cotton seed).

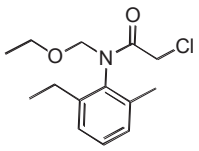
There is no obvious candidate compound for use as a residue definition for compliance, nor is there a small group of compounds that combined could usefully be used to monitor compliance. It is noted that the majority of the residue in crops can be classified according to the aniline class formed on base hydrolysis. As such a common moiety residue definition would allow residues to be monitored in all crops and derived commodities.

The major aniline metabolite class observed in maize (PE and PO) is EMA followed by OH, in soya bean commodities EMA and "other" for PE soya bean forage, HEMA and EMA for PE soya bean hay and EMA for PO soya bean hay and in cotton leaves and stems EMA and HEMA.

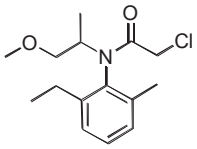
Validated analytical methods are available for the determination of compounds hydrolysable with base to EMA and HEMA in crop matrices.

Residues derived from acetochlor may also occur in rotational (follow) crops. Five metabolites, which were consistently present in plant extracts from all rotation intervals studied were: *sec*-oxanilic acid, *tert*-oxanilic acid, *sec*-sulfonic acid, *tert*-sulfonic acid, and 1-hydroxyethyl *tert*-oxanilic acid. The major aniline metabolite class in rotational crop types studied was EMA except wheat grain for which it was HMEA and HEMA.

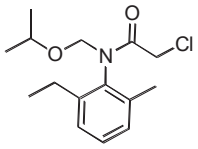
The Meeting also noted that acetochlor is a member of the chloroacetamide herbicides, a group that also includes metolachlor and propisochlor. The structures of these herbicides are similar to acetochlor and they are expected to share a number of common metabolites on cleavage of the ether side-chain.



Acetochlor

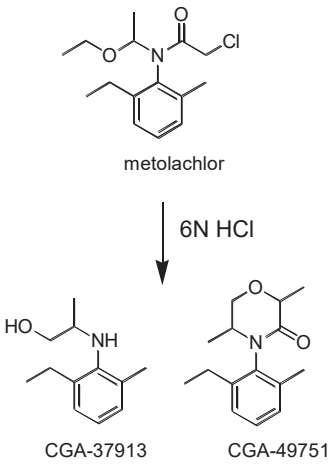


Metolachlor



Propisochlor

A common moiety method of analysis has been developed for metolachlor that involves hydrolysis in 6N HCl. The resulting compounds differ from those produced by acetochlor and where required re-analysis of samples using the metolachlor method could be used to distinguish acetochlor from metolachlor residues.



No naturally occurring compounds hydrolysable to EMA and HEMA have been identified in crops likely to be treated or grown as follow crops.

The Meeting decided the residue definition for compliance with MRLs and estimation of dietary intake in plants should be the sum of compounds converted to EMA and HEMA, expressed in terms of acetochlor

Livestock may be exposed to acetochlor-derived residues present in feeds. Due to the extensive metabolism of acetochlor in plants, exposure to unchanged parent compound is not expected. Additionally the extensive metabolism combined with metabolite profiles that differ with application type (pre-emergence or post-emergence) and also crops complicate the choice of metabolite mixtures that might usefully typify the metabolite profiles present in feed, and therefore the nature of residues in livestock commodities. Available studies involving a limited number of plant metabolites suggest the major components of the residues in livestock commodities are the dosing compounds. Therefore, as for plant commodities, it is proposed the residue definition for compliance in animals be compounds converted to EMA and HEMA. Analytical methods are available for animal matrices.

Residues hydrolysable to EMA and HEMA and captured by the residue definition are comprised of a range of hydroxylated acetochlor-derived compounds as well as conjugates, all reactions that are expected to increase water solubility. Taken as a whole, the Meeting considered that residues encompassed by the residue definition for acetochlor are not fat soluble.

Based on the above the Meeting decided the residue definition for compliance with MRLs and estimation of dietary intake should be as follows:

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

Sum of compounds converted to EMA and HEMA, expressed in terms of acetochlor.

The residue is not fat soluble.

Results of supervised residue trials on crops

Supervised residue trial data for were available for acetochlor on maize, sweet corn, cotton, sorghum, soya bean, sugar beet and peanuts. With the exception of one series of trials on maize where 5-hydroxy *sec*-oxanilic acid was analysed, residues were measured as compounds hydrolysable with base to EMA and HEMA. Residues listed below are for the sum of compounds hydrolysed to EMA and HEMA expressed in acetochlor equivalents.

The following indicates how the residues were combined when residues were reported as < LOQ for one or both of the components.

EMA	HEMA	Total residues (EMA+HEMA)
< 0.05	< 0.05	< 0.1
0.1	< 0.05	< 0.15
0.1	0.06	0.16

Sweet corn

The Meeting received supervised residue trial data for acetochlor on sweet corn from the USA. GAP in the USA is applications pre-plant or pre-emergence at up to 3.0 kg ai/ha with a PHI not required. The maximum rate per year is 3.4 kg ai/ha. In trials approximating critical GAP in the USA residues in sweet corn were (n=14): < 0.04 (14) mg/kg (kernels with husks removed).

The Meeting estimated a maximum residue level, STMR and HR or 0.04 (*), 0.04 and 0.04 mg/kg respectively for sweet corn (corn-on-the-cob).

Soya bean

In the USA acetochlor is approved for use on soya beans. GAP in the USA is applications pre-plant, pre-emergence or post-emergence but before the R2 growth stage (full flowering) at up to 1.7 kg ai/ha with a PHI not required. The maximum rate per year is 3.4 kg ai/ha. None of the trials matched critical GAP (2× 1.7 kg ai/ha post-emergence applications) and none were suitable for applying the proportionality approach.

Sugar beet

Supervised residue trial data for acetochlor on sugar beet were made available. GAP in the USA is applications pre-plant, pre-emergence or post-emergence (2 to 8 leaf stage) at up to 1.7 kg ai/ha with a PHI of 70 days. The maximum rate per year is 3.4 kg ai/ha. In trials approximating critical GAP in the USA residues in sugar beet roots were (n=15): < 0.008, < 0.009, 0.011, 0.011, 0.015, 0.016, 0.017, 0.018, 0.019, 0.021, 0.021, 0.025, 0.045, 0.051 and 0.086 mg/kg.

The Meeting estimated a maximum residue level and STMR of 0.15 and 0.018 mg/kg respectively for sugar beet roots.

Maize

The Meeting received supervised residue trial data for acetochlor on maize. GAP in the USA is applications pre-plant, pre-emergence or post-emergence (28 cm height) at up to 3.0 kg ai/ha with a PHI not specified for the EC formulation and pre-plant, pre-emergence or post-emergence (76 cm height) at up to 2.5 kg ai/ha for the CS formulation. The maximum rate per year is 3.4 kg ai/ha. The Meeting considered trials with the CS formulation where the last application can be made closer to harvest but at a lower rate compared to the EC trials where applications are made earlier but at a higher rate to give rise to higher residues and represent critical GAP. Critical GAP was considered to be pre-emergent application at 0.9 kg ai/ha followed by post-emergence application at 2.5 kg ai/ha. In trials with the CS formulation, maize was treated with a single post-emergence application rate at

approximately 3.2 kg ai/ha (1.28× the maximum label rate). The Meeting agreed to utilise the proportionality approach to estimate residues matching *c*GAP noting that residues from pre-emergence applications do not contribute to final residues and that a single post-emergence application at 2.5 kg ai/ha should be targeted for use in estimating maximum residue levels. The following scaled residues (n=21) matched *c*GAP:

Trial application rate (kg ai/ha)	Scaling factor = 2.5/trial application rate	Trial residue (mg/kg)	Scaled residue =scaling factor × trial residue (mg/kg) [a]
3.31	0.755	< 0.002	< 0.002
3.19	0.784	< 0.002	< 0.002
3.19	0.784	< 0.002	< 0.002
3.22	0.776	< 0.002	< 0.002
3.15	0.794	0.003	< 0.002
3.19	0.784	0.003	< 0.002
3.18	0.786	0.003	< 0.002
3.16	0.791	0.003	< 0.002
3.26	0.767	0.003	< 0.002
3.17	0.789	0.003	< 0.002
3.09	0.809	0.003	< 0.002
3.18	0.786	0.004	< 0.003
3.19	0.784	0.004	< 0.003
3.14	0.796	0.006	0.005
3.33	0.751	0.006	0.005
3.33	0.751	0.008	0.006
3.13	0.799	0.008	0.006
3.17	0.789	0.009	0.007
3.27	0.765	0.009	0.007
3.24	0.772	0.009	0.007
3.32	0.753	0.019	0.014

[a] LOQ for combined residues is 0.002 mg/kg.

The Meeting estimated a maximum residue level, STMR and HR of 0.02 and 0.002 mg/kg respectively for maize.

Sorghum

Acetochlor is approved in the USA for use on sorghum. GAP in the USA is applications pre-plant, pre-emergence or post-emergence (28 cm height) at up to 2.5 kg ai/ha with a PHI not specified. The maximum rate per year is 3.4 kg ai/ha. No trials matched cGAP (2× 1.7 kg ai/ha POST) and the data were not suitable for use of the proportionality approach.

Cotton

The Meeting received supervised residue trial data for acetochlor on cotton. GAP in the USA is applications pre-plant, pre-emergence or post-emergence (before 1st bloom) at up to 1.7 kg ai/ha with a PHI not specified. The maximum rate per year is 3.4 kg ai/ha. Two-post-emergence applications made closest to the latest growth stage permitted lead to highest residues. No trials utilising post-emergence application matched critical GAP in the USA.

Peanut

Supervised residue trial data for acetochlor on peanuts were available. GAP in the USA is applications pre-plant, pre-emergence or post-emergence (before flowering) at up to 1.7 kg ai/ha with a PHI not specified. The maximum rate per year is 3.4 kg ai/ha. In trials conducted in the USA plots were treated pre-plant and post-emergence (1.7 PP + 1.7 PO kg ai/ha), pre-emergent and post-emergent (1.7 PE + 1.7 POST kg ai/ha) or post-emergent (3.4 PO kg ai/ha). No trials matched cGAP (2× 1.7 PO kg ai/ha) and the data were not suitable for use of the proportionality approach.

Animal feeds

Peanut fodder

GAP in the USA is applications pre-plant, pre-emergence or post-emergence (before flowering) at up to 1.7 kg ai/ha with a PHI not specified. The maximum rate per year is 3.4 kg ai/ha. GAP in the USA is to allow a minimum of 90 days between last application and grazing or harvest and feeding of peanut hay to livestock. No trials matched *c*GAP (2× 1.7 POST kg ai/ha).

Soya bean forage

In the USA there are restraints on the grazing and feeding of post-emergence treated soya bean forage to livestock.

Soya bean fodder

In the USA acetochlor is approved for use on soya beans. GAP in the USA is applications pre-plant, pre-emergence or post-emergence but before the R2 growth stage (full flowering) at up to 1.7 kg ai/ha with a PHI not required. None of the trials matched *c*GAP and none were suitable for use of the proportionality approach.

Corn and maize forage

GAP in the USA is applications pre-plant, pre-emergence or post-emergence (28 cm height) at up to 3.0 kg ai/ha with a PHI not specified for the EC formulation and pre-plant, pre-emergence or post-emergence (76 cm height) at up to 2.5 kg ai/ha for the CS formulation. The maximum rate per year is 3.4 kg ai/ha. GAP for maize (field corn) in the USA requires that treated areas are not grazed and treated forage not fed to livestock for 40 days following application. No trials matched *c*GAP.

GAP in the USA for sweet corn is applications of an EC formulation pre-plant or pre-emergence at up to 3.0 kg ai/ha with a PHI not required. The maximum rate per year is 3.4 kg ai/ha. Residues in sweet corn forage from field trials performed in the USA approximating cGAP in the USA were (n=13): < 0.04, < 0.06, < 0.08, 0.08, < 0.09, 0.1, < 0.12, 0.14, 0.22, 0.24, 0.29, 0.44 and 0.97 mg/kg (on an as received basis). Sweet corn forage contains approximately 48% DM.

The Meeting estimated median and highest residues of 0.25 and 2.02 mg/kg for sweet corn forage (on a dry matter basis).

Corn and maize fodder

For maize (field corn), GAP in the USA is applications pre-plant, pre-emergence or post-emergence (28 cm height) at up to 3.0 kg ai/ha with a PHI not specified for the EC formulation and pre-plant, pre-emergence or post-emergence (76 cm height) at up to 2.5 kg ai/ha for the CS formulation. The maximum rate per year is 3.36 kg ai/ha. The Meeting considered trials with the CS formulation where the last application can be made closer to harvest but at a lower rate compared to the EC trials where applications are made earlier but at a higher rate to give rise to higher residues and represent critical GAP. Critical GAP was considered to be pre-emergent application at 0.9 kg ai/ha followed by post-emergence application at 2.5 kg ai/ha. No trials matched cGAP.

Residues in sweet corn fodder from field trials performed in the USA approximating cGAP in the USA were (n=14): < 0.04, < 0.04, < 0.04, < 0.04, 0.04, < 0.05, < 0.06, 0.08, 0.09, 0.10, 0.13, 0.13, 0.42 and 0.91 mg/kg (on an as received basis). Sweet corn fodder contains approximately 83% DM.

The Meeting estimated a maximum residue level and median and highest residues of 1.5, 0.07 and 0.91 mg/kg for sweet corn fodder (on as received matter basis) or 1.5, 0.084 and 1.096 mg/kg (dry matter basis) assuming 83% dry matter (DM).

Sorghum forage

GAP in the USA requires that treated areas are not grazed and treated forage not fed to livestock for 60 days following application. In the USA applications are made pre-plant, pre-emergence or post-emergence (28 cm height) at up to 2.5 kg ai/ha. No trials matched *c*GAP.

Sorghum fodder (stover)

GAP in the USA is applications pre-plant, pre-emergence or post-emergence (28 cm height) at up to 2.5 kg ai/ha with a PHI not specified. No trials matched cGAP.

Cotton gin by-products

No trials on cotton matched GAP in the USA.

Sugar beet tops

GAP in the USA is applications pre-plant, pre-emergence or post-emergence (2 to 8 leaf stage) at up to 1.7 kg ai/ha with a 70 day interval between the last application and grazing or harvest of sugar beet tops. The maximum seasonal application is 3.4 kg ai/ha/year. Residues in sugar beet tops from field trials performed in the USA approximating cGAP in the USA were (n=15): 0.009, 0.014, 0.019, 0.028, 0.028, 0.030, 0.035, 0.041, 0.043, 0.050, 0.051, 0.056, 0.063, 0.147 and 0.554 mg/kg (on an as received basis). Sugar beet tops contain approximately 23% DM.

The Meeting estimated a maximum residue level and median residues of 3 and 0.178 mg/kg for sugar beet tops (on dry matter basis).

Rotational crop residues

Soil residues of acetochlor related compounds are moderately persistent. The use-pattern (USA GAP) specifies plant-back intervals for certain follow-crops as well as crops that may be rotated following application:

- Non-grass animal feeds such as alfalfa, clover, kudzu, lespedeza, lupin, sainfoin, trefoil, velvet bean, and Vetch spp. may be planted 9 months (270 days) after application.
- Wheat may be planted 4 months (120 days) after application.
- Rotate the next season to the following crops—soya beans, corn (all types), milo (sorghum), cotton, sugar beets, sunflowers, potatoes, barley, buckwheat, millet (pearl and proso), oats, rye, teosinte, triticale, wild rice, dried shelled bean group Lupinus spp. (including grain lupin, sweet lupin and white lupin), Phaseolus spp. (includes field beans, kidney beans, lima beans (dry), navy beans, pinto bean and tepary beans), bean Vigna spp. (includes adzuki beans, black-eyed peas, catjang, cowpeas, Crowder peas, moth beans, mung beans, rice beans, southern peas and urd beans), broad beans (dry), chickpeas, guar, lab lab beans, lentils, peas (Pisum spp., includes field peas) and pigeon peas.

Field crop rotation residue trials are available for representative crops that may be rotated. In these trials follow crops were planted after harvesting of maize that had been treated with acetochlor as a pre-plant, pre-emergence or seed treatment at 3.4 kg ai/ha equivalent to the maximal seasonal rate in the USA. The Meeting considered these trials reflect likely residues in crops grown in rotation following application at the maximum seasonal rate (3.4 kg ai/ha/year).

Legume animal feed as a follow crop

Residues in follow crops of alfalfa and clover as representative legume feed commodities were made available to the Meeting. Alfalfa was sown 274–355 days after pre-emergent application to maize. Clover was sown 274–355 days after pre-emergent application to maize. Residues are listed below:

Alfalfa forage (n=17): < 0.04, 0.04, 0.06, 0.07, 0.08, 0.08, 0.08, 0.09, 0.11, 0.14, 0.14, 0.16, 0.20, 0.29, 0.35, 0.47 and 0.54 mg/kg (fresh weight basis).

Clover forage (n=18): < 0.03, < 0.04, < 0.04, 0.04, < 0.05, < 0.05, 0.05, 0.06, 0.10, 0.10, 0.10, 0.10, 0.11, 0.16, 0.17, 0.17, 0.35 and 0.57 mg/kg (fresh weight basis).

The Meeting estimated median and highest residues in legume forage of 0.10 and 0.57 mg/kg (as received basis) or 0.333 and 1.9 mg/kg when expressed on a dry matter basis (assuming 35%DM for alfalfa and 30%DM for clover).

Alfalfa hay (n=16): 0.11, 0.14, 0.15, 0.16, 0.18, 0.19, 0.20, 0.24, 0.28, 0.29, 0.33, 0.34, 0.73, 0.82, 0.97, and 1.87 mg/kg (fresh weight basis).

Clover hay (n=17): < 0.02, < 0.02, < 0.04, 0.08, 0.08, 0.08, 0.12, 0.13, 0.13, 0.15, 0.24, 0.30, 0.41, 0.44, 0.48, 0.76 and 1.24 mg/kg (fresh weight basis).

The median residues in the clover and alfalfa hay datasets differed by less than a factor of five and the Meeting decided to recommend a group maximum residue level for legume animal feeds. In deciding which data set to use for the recommendation, as a Mann Whitney U-test indicated that the residue populations were not different it was decided to combine the data sets.

Residues in alfalfa and clover fodder (hay) of follow crops ranged from < 0.02 to 1.87 mg/kg (as received basis). Alfalfa and clover hay contains approximately 89%DM.

The Meeting estimated maximum residue levels, median and highest residues of [2, 0.20 and 1.87 mg/kg fresh weight basis] 3, 0.225, and 2.101 mg/kg (dry matter basis) for legume animal feeds.

Wheat (forage, straw, grain)

Wheat may be planted as a follow crop four months after application. Residues (as received basis) in follow wheat crops planted 90–176 days after pre-emergent application to maize were:

- Forage (n=18): < 0.02, < 0.02, < 0.02, < 0.03, < 0.03, 0.03, 0.04, < 0.05, 0.06, 0.06, 0.11, 0.13, 0.14, 0.18, 0.19, 0.27, 0.41 and 0.47 mg/kg (fresh weight basis).

The Meeting estimated median and highest residues in wheat forage of 0.06 and 0.47 mg/kg (fresh weight basis) or 0.24 and 1.88 mg/kg when expressed on a dry matter basis (assuming 25%DM).

- Straw (n=18): < 0.02, < 0.02, < 0.02, < 0.02, < 0.02, < 0.02, < 0.02, 0.03, 0.03, 0.03, 0.04, 0.07, 0.07, 0.07, 0.07, 0.08, 0.09 and 0.10 mg/kg.

The Meeting estimated maximum residue levels, median and highest residues of 0.2, 0.034, and 0.114 mg/kg for wheat straw and fodder (dry matter basis) assuming wheat straw contains 88% dry matter.

- Grain (n=18): < 0.02 (18) mg/kg.

The Meeting estimated maximum residue levels and median residues of 0.02 (*) and 0.02 mg/kg for wheat grain.

Other cereals (forage, hay, straw, grain)

In the USA, a number of cereal and grass-like crops (other than wheat, maize, sorghum) may be planted approximately one year after last application. Residues (on an as received basis) in follow oat crops planted the next season after pre-emergent application to maize:

- Forage (n=18): < 0.035 (7), < 0.038, < 0.038, < 0.042, 0.048, 0.056, 0.057, 0.063, 0.066, 0.085 and 0.121 mg/kg (fresh weight basis).

The Meeting estimated median and highest residues in oat forage of 0.04 and 0.121 mg/kg (as received basis) or 0.13 and 0.40 mg/kg when expressed on a dry matter basis (assuming 30%DM).

- Hay (n=16): < 0.035 (6), < 0.036, ≤ 0.036, ≤ 0.042, 0.042, 0.060, 0.068, 0.074, 0.091, < 0.098 and 0.156 mg/kg (fresh weight basis).

The Meeting estimated median and highest residues of 0.039, and 0.173 mg/kg for oat hay (dry matter basis).

- Straw (n=17): < 0.035 (11), < 0.036, < 0.036, 0.044, 0.044, 0.070, and 0.254 mg/kg (fresh weight basis).

The Meeting estimated maximum residue levels, median and highest residues of 0.3, 0.039, and 0.282 mg/kg for oat straw (dry matter basis) assuming straw contains 90% dry matter.

- Grain (n=17): < 0.035 (16) and < 0.036 mg/kg.

The Meeting estimated a maximum residue level and STMR of 0.04 (*) and 0.035 mg/kg for oat grain.

The Meeting agreed to extrapolate the results for oats to other cereals that are permitted in the USA as follow crops and not treated directly—barley, buckwheat, millet (pearl and proso), rye, teosinte, triticale and wild rice commodities. The Meeting decided not to extrapolate the results to follow rice crops as the cultivation practices for rice differ from those of other cereal crops and this may impact on residues.

Sunflowers

Sunflowers are a permitted follow crop when planted the following year. Residues in seed of follow sunflower crops planted 350–384 days after pre-emergent application to maize were all < 0.04 (8) mg/kg. The Meeting estimated a maximum residue level and STMR of 0.04 (*) and 0.04 mg/kg for sunflower seed.

Potato

Potatoes are a permitted follow crop when planted the following year. Residues in tubers of follow potato crops (planted 291–380 days after pre-emergent application to maize) were all < 0.04 (10) mg/kg. The Meeting estimated a maximum residue level, STMR and HR of 0.04 (*), 0.04, and 0.04 mg/kg for potatoes.

Beans (dry), Peas (dry)

A number of legume grains are permitted follow crops to be planted the next season (about one year after the last application). Residues in grain of follow bean and pea crops were all < 0.02 mg/kg, nine bean trials and five pea trials. The Meeting estimated maximum residue levels of 0.02 (*) for beans and peas (dry) and STMRs or 0.02 mg/kg. The two maximum residue levels would cover residues in follow *Phaseolus* spp as well as *Vigna* spp and *Pisum* spp.

Rotational crop trials were available for follow soya beans. The observed residues are higher than reported for beans and peas (dry) and residues in follow soya beans could be used as a representative crop for the remaining pulses permitted to be rotated in the USA—*Lupinus* spp., broad beans, chickpeas, Hyacinth beans (lab lab beans), lentils, and pigeon peas.

Residues in seed of follow soya beans were (n=16): ≤ 0.02 (8), 0.02, < 0.03, 0.03, 0.03, 0.04, 0.04, 0.06 and 0.10 mg/kg.

The Meeting agreed to extrapolate to residues in seed of follow soya bean to *Lupinus* spp., broad beans (dry), chickpeas, Hyacinth beans, dry (lab lab beans), lentils, and pigeon peas and estimated maximum residue limits of 0.15 and STMRs of 0.02 mg/kg for these seeds.

Fate of residues during processing

The Meeting received information on the fate of incurred residues of acetochlor during the processing of soya beans, sugar beets, sorghum, cotton, peanuts and sunflower seeds. A study of the nature of the

residue of acetochlor under simulated processing conditions (pasteurization, baking/brewing/boiling, sterilization) showed acetochlor, if present, is stable.

Summaries of relevant acetochlor processing factors are provided below.

	Processed Fraction	Processing Factor	Best estimate PF	RAC STMR or median	STMR × PF = STMR-P	RAC HR or highest	HR × PF = HR-P
Sugar beet	Dried pulp	2.3, 0.9	1.6	0.018	0.029	0.086	0.138
	Molasses	4.2, 1.1	2.65		0.048		0.228
	Refined sugar	0.5, < 0.25	0.375		0.0068		0.032
Sunflower	Meal	1.4	1.4	0.04	0.056	0.04	0.056
	Oil	0.22	0.22		0.0088		0.0088

PFs are based on combined EMA and HEMA aniline class metabolites: PFs calculated as EMA + HEMA, expressed as acetochlor in processed commodity divided by EMA + HEMA in the RAC

The Meeting recommended a maximum residue level of 0.3 mg/kg for sugar beet molasses and a median residue of 0.048 mg/kg. For sugar beet pulp (dry) the Meeting recommended a maximum residue level of 0.3 mg/kg and a median residue of 0.029 mg/kg.

Residues in animal commodities

Farm animal feeding studies

The Meeting received information on the residue levels in tissues and milk of dairy cows dosed with a mixture of four EMA class acetochlor plant metabolites (*tert*-hydroxy and the sodium salts of *tert*-sulfonic acid, *tert*-oxanilic acid and *tert*-sulfinylacetic acid and present in equal proportions) at the equivalent of 5, 15 and 50 ppm acetochlor equivalents in the feed for 28 consecutive days. Based on HPLC retention times for extracts in plant metabolism studies, it is concluded that the properties of the dosing compounds encompass the range of polarities of the majority of compounds observed in the plant metabolism studies (log K_{ow} of dosing compounds ranged from –3.2 to 2.2). The studies are considered to cover the likely transfer of acetochlor-related residues, including those from different aniline metabolite classes, from feed to livestock.

Residues in milk were < 0.02 mg/kg (acetochlor equivalents) for the 50 ppm dose group for all sample intervals.

In kidney mean residues were < 0.02, 0.03, and 0.07 mg/kg (acetochlor equivalents) for the 5, 15, and 50 ppm dose groups respectively. Mean residues liver residues were < 0.02 and 0.02 mg/kg for the 15 and 50 ppm dose groups while mean residues in fat and muscle were < 0.02 mg/kg for all samples in the 50 ppm dose group. As no residues were observed at the highest dose level samples muscle and fat from other dose groups were not analysed.

Laying hens dosed at the equivalent of 5, 15 and 50 ppm acetochlor with a mixture of *tert*-hydroxy and the sodium salts of *tert*-sulfonic acid, *tert*-oxanilic acid and *tert*-sulfinylacetic acid for 28 days. No residues above the LOQ were detected in any tissues or eggs, LOQ 0.05 mg/kg for kidney and LOQ 0.02 mg/kg for other tissues and eggs.

Estimation of livestock dietary burdens

Dietary burden calculations for beef cattle, dairy cattle and poultry are provided below. The dietary burdens were estimated using the OECD diets listed in Appendix IX of the 2009 edition of the FAO Manual.

Potential cattle feed items include legume fodder, cereal forage and fodder, sugar beet tops and various grains.

Summary of livestock dietary burden (ppm acetochlor equivalents of dry matter diet)

	US-Canada		EU		Australia		Japan	
	max	mean	Max	mean	max	Mean	max	Mean
Beef cattle	0.4	0.1	2.0	0.3	2.1	0.3	0.2	0.03
Dairy cattle	1.4	0.2	1.6	0.2	2.1 [a, b]	0.3 [c, d]	0.6	0.09
Broilers	0.03	0.02	0.05	0.04	0.02	0.02	0.1	0.03
Layers	0.03	0.02	0.54 [e]	0.10 [f]	0.02	0.02	0.01	0.01

[a] Highest maximum beef or dairy cattle dietary burden suitable for MRL estimates for mammalian meat

[b] Highest maximum dairy cattle dietary burden suitable for MRL estimates for mammalian milk

[c] Highest mean beef or dairy cattle dietary burden suitable for STMR estimates for mammalian meat

[d] Highest mean dairy cattle dietary burden suitable for STMR estimates for milk

[e] Highest maximum poultry dietary burden suitable for MRL estimates for poultry meat and eggs

[f] Highest mean poultry dietary burden suitable for STMR estimates for poultry meat and eggs

Animal commodity maximum residue levels

The calculations used to estimate highest total residues for use in estimating maximum residue levels, STMR and HR values are shown below.

	Feed level	Residues	Feed level	Residues (mg/kg) in			
	(ppm) for milk residues	(mg/kg) in milk	(ppm) for tissue residues	Muscle	Liver	Kidney	Fat
MRL beef or dairy cattle							
Feeding study [a]	5	< 0.02	15	< 0.02	< 0.02	0.04	< 0.02
Dietary burden and high residue	2.1	< 0.008	2.1	< 0.003	< 0.003	0.0056	< 0.003
STMR beef or dairy cattle							
Feeding study [b]	5	< 0.02	15	< 0.02	< 0.02	0.03	< 0.02
Dietary burden and median residue estimate	0.3	< 0.0012	0.3	< 0.0004	< 0.0004	0.0006	< 0.0004

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

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

The Meeting estimated the following maximum residue levels: milk 0.02* mg/kg; meat (mammalian except marine mammals) 0.02* mg/kg, mammalian fat (except milk fat) 0.02* mg/kg and edible offal 0.02* mg/kg.

For poultry no residues were observed in eggs and tissues on dosing laying hens at up to 50 ppm in the diet for 28 days. The Meeting estimated the following maximum residue levels for poultry commodities: poultry meat 0.02* mg/kg; poultry edible offal 0.02* mg/kg and eggs 0.02* mg/kg. The Meeting estimated the following STMR and HR values: poultry meat 0 mg/kg; poultry fat 0 mg/kg; poultry edible offal 0 mg/kg and eggs 0 mg/kg.

RECOMMENDATIONS FURTHER WORK OR INFORMATION

On the basis of the data obtained from supervised residue 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 compliance with MRL and estimation of dietary intake (for animal and plant commodities):

Sum of compounds hydrolysable with base to 2-ethyl-6-methylaniline (EMA) and 2-(1-hydroxyethyl)-6-methylaniline (HEMA), expressed in terms of acetochlor.

The residue is not fat soluble.

DIETARY RISK ASSESSMENT

Long-term intake

The 2015 JMPR established an Acceptable Daily Intake (ADI) of 0–0.01 mg/kg bw for acetochlor.

The evaluation of acetochlor resulted in recommendations for MRLs and STMR values for raw and processed commodities. Where data on consumption were available for the listed food commodities, dietary intakes were calculated for the 17 GEMS/Food Consumption Cluster Diets. The results are shown in Annex 3 to the 2015 Report.

The IEDIs in the seventeen Cluster Diets, based on the estimated STMRs were 0–4% of the maximum ADI (0.01 mg/kg bw). The Meeting concluded that the long-term intake of residues of acetochlor from uses that have been considered by the JMPR is unlikely to present a public health concern.

Short-term intake

The 2015 JMPR established an Acute Reference Dose (ARfD) of 1 mg/kg bw for acetochlor. The IESTI of acetochlor for the commodities for which STMR, HR and maximum residue levels were estimated by the current Meeting are shown in Annex 4 to the 2015 Report. The IESTI represented 0–0% of the ARfD.

The Meeting concluded that the short-term intake of residues of acetochlor resulting from uses that have been considered by the JMPR is unlikely to present a public health concern.