ACETOCHLOR

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Explanation

Acetochlor is the International Organization for Standardization (ISO)–approved common name for 2-chloro-*N*-(ethoxymethyl)-*N*-(2-ethyl-6-methylphenyl)acetamide (International Union of Pure and Applied Chemistry), with Chemical Abstracts Service 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 the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) and was reviewed by the present Meeting at the request of the Codex Committee on Pesticide Residues.

All studies evaluated in this monograph were performed by laboratories that were certified for good laboratory practice (GLP) and that complied, where appropriate, with the relevant Organisation for Economic Co-operation and Development (OECD) test guidelines or similar guidelines of the European Union or United States Environmental Protection Agency, unless otherwise indicated.

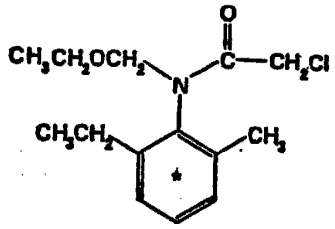
Evaluation for acceptable intake

1. Biochemical aspects

The absorption, distribution, metabolism and excretion of acetochlor were studied in rats following a single oral low dose, a single oral high dose and a single oral low daily dose repeated for 14 days followed by a radioactive dose.

Fig. 1 shows the structure of [phenyl-U-^{14}C]acetochlor.

Fig. 1. Structure of [phenyl-U-^{14}C]acetochlor



* Denotes position of ^{14}C label.

1.1 Absorption, distribution and excretion

(a) Oral administration

In an absorption, distribution and excretion study, CD Sprague-Dawley rats (five of each sex) were administered [phenyl-U-^{14}C]acetochlor (purity at least 97.6%) in a single oral gavage dose of 10 mg/kg body weight (bw) in polyethylene glycol 400. A further five male and five female rats were dosed at 10 mg/kg bw for the determination of plasma concentrations at various intervals up to 240 hours.

Less than 0.15% of the administered dose was excreted in carbon dioxide in the preliminary study, so carbon dioxide was not collected in the main study. Over 5 days, males excreted 70.6% of the dose in urine and 22.8% of the dose in faeces. Females eliminated 77.0% in urine and 13.2% in faeces (Table 1). Over half of the administered dose was excreted within 24 hours after dosing (59.5% in males and 66.5% in females). The majority of the excretion in faeces occurred in the first 48 hours. The radioactivity retained in tissues and organs accounted for 3.9% of the administered dose on day 5 in males and 3.7% in females. The whole blood sample taken at termination contained 1.8% and 2.0% of the dose for males and females, respectively. The remaining carcass contained about 0.7% of the administered dose. Tissue concentrations after 5 days were highest in whole blood (4.5–6.2 µg acetochlor equivalents/g), but the very low levels in plasma showed that the radioactivity was associated with the cellular fraction of blood. Much lower concentrations – approximately 0.5 µg equivalents/g or lower – were present in spleen, heart, lungs and liver.

Table 1. Recoveries of administered radioactivity over 5 days after administration of ^{14}C-labelled acetochlor in a single oral dose of 10 or 200 mg/kg bw or a single dose of 10 mg/kg bw following 14 daily oral doses of non-radiolabelled acetochlor at 10 mg/kg bw

	% of administered radioactivity excreted over 5 days (mean of five rats)					
	10 mg/kg bw[a]		200 mg/kg bw[b]		10 mg/kg bw repeated for 14 days followed by a radioactive dose[c]	
	Males	Females	Males	Females	Males	Females
Urine	69.8	75.8	51.1	63.8	64.3	73.7
Faeces	22.8	13.2	37.2	26.9	26.1	16.5
Cage wash	0.8	1.2	0.5	0.9	0.7	0.9
Tissues/organs	3.9	3.7	4.1	3.5	3.2	3.5
Total recovered	97.3	94.0	97.3	95.1	94.3	94.6

bw: body weight
[a] Data extracted from Hawkins, Kirkpatrick & Dean (1988)
[b] Data extracted from Hawkins, Kirkpatrick & Dean (1989a).
[c] Data extracted from Hawkins, Kirkpatrick & Dean (1989b).

The mean plasma concentrations reached peaks at 7 hours after dosing in both male (1.27 ± 0.32 µg/g) and female (1.62 ± 0.09 µg/g) rats. The plasma concentrations were consistently higher in females than in males. Mean concentrations appeared to decline biphasically (Hawkins, Kirkpatrick & Dean, 1988).

In an absorption, distribution and excretion study, CD Sprague-Dawley rats (five of each sex) were administered [phenyl-U-^{14}C]acetochlor (purity > 98.9%) in a single oral gavage dose of 200 mg/kg bw in polyethylene glycol 400. A further five male and five female rats were dosed at 200 mg/kg bw for the determination of plasma concentrations at various intervals up to 360 hours.

Over 5 days, males excreted 51.6% of the dose in urine and 37.2% of the dose in faeces. Females eliminated 64.7% in urine and 26.9% in faeces. Over half of the administered dose was excreted within 24 hours after dosing (43.1% in males and 51.7% in females) (Table 1). The majority of the excretion in faeces occurred in the first 48 hours. The radioactivity retained in tissues and organs accounted for 4.1% of the administered dose at day 5 in males and 3.5% in females. The whole blood sample taken at sacrifice contained 2.1% and 1.9% of the dose for males and females, respectively. The remaining carcass contained a mean of 0.9% of the administered dose for males and 0.7% for females. Tissue concentrations after 5 days were highest in whole blood (112 and 105 µg acetochlor equivalents/g for males and females, respectively). The mean ratios of whole blood to plasma concentrations were 140 and 110 for males and females, respectively. The next higher concentrations were found in heart, lungs, kidneys and liver (about 7.6–23 µg/g).

The mean plasma concentrations reached peaks at 12 hours after dosing in both male (25.0 ± 4.6 µg/g) and female (41.9 ± 9.6 µg/g) rats. The plasma concentrations were consistently higher in females than in males. Mean concentrations appeared to decline biphasically (Hawkins, Kirkpatrick & Dean, 1989a).

In a repeated-dose absorption, distribution and excretion study, [phenyl-U-^{14}C]acetochlor (purity > 98%) was administered in a single oral gavage dose of 10 mg/kg bw in polyethylene glycol 400 to CD Sprague-Dawley rats (five of each sex) that had previously been administered non-radiolabelled acetochlor (purity not reported) in 14 daily oral doses of 10 mg/kg bw.

Over 5 days, males excreted 65.0% of the dose in urine and 26.1% of the dose in faeces. Females eliminated 74.6% in urine and 16.5% in faeces (Table 1). Over half of the administered dose was excreted within 24 hours after dosing (57.2% in males and 67.1% in females). The majority of the excretion in faeces occurred in the first 48 hours. The radioactivity retained in tissues and organs accounted for 3.2% of the administered dose at day 5 in males and 3.5% in females. The whole blood sample taken at sacrifice contained 1.5% and 1.9% of the dose for males and females, respectively. The remaining carcass contained about 0.6% of the dose for males and females. Tissue concentrations after 5 days were highest in whole blood (4.41 and 4.72 µg acetochlor equivalents/g for males and females, respectively). The mean ratios of whole blood to plasma concentrations were 100 and 98 for males and females, respectively. The next highest concentrations were found in heart, spleen, lungs, kidneys and liver (about 0.38–1.1 µg/g).

In this repeated-dose study, acetochlor was well absorbed and rapidly excreted, with no pronounced sex differences. The toxicokinetics of acetochlor were not modified by a repeated dose and were almost the same as after either a high or a low single dose (Hawkins, Kirkpatrick & Dean, 1989b).

A study was designed to determine the overall tissue distribution and localization, especially in the nasal turbinate tissue, of ^{14}C-labelled acetochlor residues following oral administration to rats and mice. The nasal turbinate is a target tissue in rats. The details of this study are provided in section 2.6 on the mode of action of acetochlor.

Acetochlor and/or its metabolites were found to accumulate specifically in the olfactory region of the nasal turbinates in the rat and remained detectable for at least 7 days. No specific accumulation of acetochlor and/or its metabolites was observed in the olfactory region of the nose in mice (Kraus & Wilson, 1996).

A study was conducted to characterize the absorption and routes and rates of excretion and to determine the bioaccumulation of ^{12}C, ^{13}C and ^{14}C isotopically labelled (homogeneous mixture) acetochlor administered by oral intubation to young adult male and female Sprague-Dawley rats. The study design is shown in Table 2.

Only 0.04% of the administered dose was recovered in exhaled air in group A animals over 7 days; therefore, the radioactivity in exhaled air in groups B, C and D was not measured. Excretion of radioactivity in urine and faeces in treated groups is shown in Table 3. The cumulative rate of excretion (urine and faeces combined) in male and female rats in treated groups is shown in Table 4.

Table 2. Study design and sampling

Group	No. of rats	Dose (mg/kg bw)	Dosing regimen	Excretion period (days)	Samples for radioactivity determination
A	3 M, 3 F	400	Single dose	7	Expired air only (preliminary test)
B	5 M, 5 F	10	Single dose	7	Urine, faeces, blood, tissues and quantification of metabolites[a]
C	5 M, 5 F	400	Single dose	7	Urine, faeces, blood, tissues and quantification of metabolites[a]
D	5 M, 5 F	10	14 daily doses of cold material, then single dose of radioactive material	7	Urine, faeces, blood, tissues and quantification of metabolites[a]

bw: body weight; F: female; M: male

[a] Results of metabolite studies described in section 1.2 below.

Source: Carr et al. (1983)

Table 3. Excretion of radioactivity in various groups of acetochlor-treated rats[a]

	% of administered radioactivity excreted over 7 days					
	Group B		Group C		Group D	
	Males	Females	Males	Females	Males	Females
Urine	31.5	43.4	46.7	49.7	59.4	67.8
Faeces	50.1	40.4	28.9	26.6	25.6	19.3
Tissues/organs	3.2	3.0	3.5	3.7	2.6	2.8
Residual carcass	1.25	1.05	1.66	1.25	0.725	0.69
Cage wash	0.5	0.5	0.8	0.7	0.3	0.3
Minimum absorbed	36.5	48.0	52.7	55.4	63.0	71.6
Total excreted	81.6	83.7	75.6	76.3	85.1	87.1
Total recovered	86.6	88.2	81.6	82.0	88.7	90.9

[a] Average five rats of each sex.
Source: Carr et al. (1983)

Table 4. Cumulative rate of excretion of radioactivity (urine and faeces combined) in various groups of acetochlor-treated rats in 7 days[a]

	% of administered radioactivity excreted over 7 days					
	Group B		Group C		Group D	
Day	Males	Females	Males	Females	Males	Females
0.5	16.7	22.8	7.2	6.0	30.3	43.4
1	46.5	45.3	24.1	17.8	39.6	34.7
2	13.4	12.7	37.2	47.5	10.9	6.9
3	3.4	1.4	4.2	2.4	2.4	1.0
4	0.6	0.7	1.4	1.2	0.9	0.4
5	0.4	0.4	0.7	0.6	0.5	0.3
6	0.3	0.2	0.5	0.4	0.3	0.2
7	0.3	0.2	0.3	0.4	0.2	0.2
Total	81.6	83.7	75.6	76.3	85.1	87.1

[a] Average five rats of each sex.
Source: Carr et al. (1983).

As shown in Table 4, the combined excretion in faeces and urine of group B was 81.6% and 83.7% of the dose for males and females, respectively. The mean percentages of the administered dose retained in tissues were 3.24% and 3.04% for males and females, respectively (Table 3). The combined excretion in faeces and urine of group C was 75.6% and 76.3% of the dose for males and females, respectively (Table 4). The mean percentages of the administered dose retained in tissues were 3.50% and 3.69% for males and females, respectively (Table 3). The combined excretion in faeces and urine of group D was 85.1% and 87.1% of the dose for males and females, respectively (Table 4). The mean percentages of the administered dose retained in tissues was 2.58% and 2.79% for males and females, respectively (Table 3). Almost all excretion of the radiolabel occurred within the first 48 hours (Table 3).

Table 5. Elimination half-lives for males and females of groups B, C and D[a]

	Elimination half-life (h)			
	α phase		β phase	
Group	Males	Females	Males	Females
B	7.13	5.77	162	182
C	10.4	9.3	249	286
D	7.1	5.4	129	186

[a] Average five rats of each sex.
Source: Carr et al. (1983).

The urinary and faecal excretion combined showed biphasic elimination, where α is the rapid whole-body elimination phase and β is the slow elimination phase (Table 5).

Analysis of blood revealed higher levels of radioactivity in whole blood than in plasma. Analysis of the red cell fraction of the blood showed that the retained radioactivity was bound to haemoglobin molecules. The mean percentage of the dose retained in whole blood in all test groups ranged from 1.95% to 2.95% (Carr et al., 1983).

Twelve male and six female Sprague-Dawley rats were administered a single oral dose of ^{14}C-phenyl-labelled acetochlor at 10 or 200 mg/kg bw in polyethylene glycol 600. The bile ducts of four of the male rats (two each at 10 and 200 mg/kg bw) were cannulated 24 hours prior to dosing. The dosing regimen and samples collected are shown in Table 6.

Excretion of the 200 mg/kg bw dose of ^{14}C-labelled acetochlor in this study is shown in Table 7. These results show that the majority of the dose was excreted within 24 hours, with no marked sex differences apparent. However, the data indicate that a greater proportion of the dose was excreted via faeces compared with urine, which contrasts with excretion data for similarly dosed, but individually housed, rats in previous studies (Hawkins, Kirkpatrick & Dean, 1989a,b,c). This may be due to contamination of faeces with urine.

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. At 200 mg/kg bw, an average 80.2% of the dose was eliminated via bile, 8.5% in urine and 3.9% in faeces (Table 8). Approximately 84% of the administered dose was excreted in bile within 24 hours, indicating that the biliary route of elimination was very important (Jones, 1990).

Table 6. The dosing regimen and sampling scheme

Dose (mg/kg bw)	No. of rats	Sex	Sampling period (h)	Samples
200	8	M	72	Urine, faeces, cage wash
200	6	F	72	Urine, faeces, cage wash
10	2	M	48	Bile, urine, faeces, cage wash
200	2	M	48	Bile, urine, faeces, cage wash

bw: body weight; F: female; M: male
Source: Jones (1990)

Table 7. Excretion profiles for rats given a single oral dose of labelled acetochlor at 200 mg/kg bw

Sample	Sampling interval (h)	Males	Females
Urine	0–24	20.1	27.25
	24–48	5.28	6.46
	48–72	2.56	1.92
	0–72	27.85	35.63
Faeces	0–24	35.3	24.15
	24–48	13.15	15.87
	48–72	2.18	2.64
	0–72	50.63	42.66
Cage wash	0–72	0.5	6.62
Total recovery		78.98	84.91

bw: body weight
Source: Jones (1990)

Table 8. Mean recoveries (n = 2) of administered radioactivity in male bile duct–cannulated rats over 48 hours after a single oral dose of ^{14}C-labelled acetochlor at 10 or 200 mg/kg bw

		% of the dose	
Fraction	**Sampling interval (h)**	**10 mg/kg bw**	**200 mg/kg bw**
Urine	0–48	8.0	8.5
Bile	0–48	85.1	80.2
Faeces	0–48	4.2	3.9
Total	0–48	97.4	92.5
Bile	0–4	24.4	27.8
	4–8	29.3	18.0
	8–12	16.0	14.2
	12–24	14.3	18.1
	24–36	1.06	1.94
	36–48	0.08	0.12

bw: body weight
Source: Jones (1990)

(b) Other routes of administration

The dermal penetration of acetochlor from a topically applied formulated product containing acetochlor (Harness herbicide formulation) was investigated in rhesus monkeys. Studies were first conducted to determine the extent of urinary and faecal elimination of radioactivity following administration of ^{14}C-labelled acetochlor via three parenteral routes: intravenous, intramuscular and subcutaneous. The results of the parenteral studies were then used to correct for incomplete urinary and faecal excretion in the topical study to determine an estimate of the extent of dermal penetration of acetochlor from Harness herbicide formulation.

In this study, four male rhesus monkeys received 1 mg of radioactive acetochlor formulation in propylene glycol as the vehicle via the intravenous, intramuscular and subcutaneous routes. Urine

and faeces were collected at selected time points and analysed for radioactivity. In a percutaneous absorption study, six male rhesus monkeys each received radioactive acetochlor formulation (Harness; emulsifiable concentrate formulation) on their abdomen. Acetochlor (1119 mg) was applied to 12 cm^2 of abdominal area from which hair had been lightly clipped. After 24 hours, the application site was washed twice with soap and water. Urinary and faecal samples were collected at selected time points for 7 days.

The percentage of radioactivity excreted in faeces in 7 days was negligible. The percentages of radioactivity excreted in the urine in 7 days were 87.7%, 96.7% and 83.2% following administration by the intravenous, intramuscular and subcutaneous routes, respectively. The radioactivity in the urine in 7 days following dermal application was 9.2%, corrected for incomplete excretion via the intramuscular route. Peak time periods for the excretion of radioactivity were 0–4 hours, 0–4 hours, 4–24 hours and 36–96 hours for the intravenous, intramuscular, subcutaneous and dermal applications, respectively. For the dermal route, 7 days was not sufficient to reach background radioactivity levels. Pharmacokinetic analysis of the semi-log plot of the percentage of dose excreted in urine versus time revealed biphasic elimination (α phase and β phase) of radioactivity in urine after parenteral administration and a one-phase elimination of radioactivity in urine after dermal application. The absorption half-lives were 4.4 and 25.4 hours following subcutaneous and dermal administration, respectively. The elimination half-life for the dermal route was 92.5 hours.

In summary, the dermal absorption of acetochlor was low (9.2%) based on urinary excretion, and 7 days was not sufficient time to reach background levels of radioactivity (Maibach, 1983).

A study was conducted to determine the route and rate of elimination of acetochlor following intravenous administration to rhesus monkeys at two different doses and to characterize acetochlor metabolites in monkey urine (see section 1.2 below). A specific purpose of the study was to provide analytical information that could be used in the calculation of a systemic body burden from acetochlor residues in urine from European applicator exposure monitoring studies.

Two groups of male rhesus monkeys were administered ^{14}C-phenyl-labelled acetochlor intravenously, using propylene glycol/ethanol/saline (60:10:30) as the dose vehicle, at a dose level of 0.005 or 0.05 mg/kg bw. Three monkeys were treated at each dose level, and urine and faeces were collected at various times up to 5 days. The metabolism cages were rinsed with a minimal amount of water after each collection of urine and faeces. The first cage wash was added to the urine specimen. The second cage wash was retained separately as the cage rinse. The final cage rinse was performed with 50:50 (volume per volume) methanol/water.

Total recoveries of the administered dose ranged from 77.3% to 88.6%. Elimination of the dose was rapid for both groups, with 75.9–87.8% of the dose excreted within 48 hours. Excretion in the urine, which includes cage washes, was the major route of elimination and accounted for 76.9–84.2% of the dose for the group treated at 0.005 mg/kg bw, whereas elimination in the faeces accounted for 4.4–7.6% of the dose. For the group treated with 0.05 mg/kg bw, the amount excreted in the urine ranged from 72.8% to 76.1%, whereas the amount eliminated in the faeces ranged from 3.7% to 5.2%. The data also show that the majority of the radioactivity excreted in urine was excreted within the first 6 hours and that the total amount excreted in urine (> 98%) was excreted within the first 48 hours for all animals in each group. Based on the similarity between the two dose groups, an average of 77.2% of the administered dose was excreted in the urine, and the study author suggested that a correction factor of 77.2% should be used for European applicator exposure monitoring studies for acetochlor (Kurtzweil, 2014).

1.2 Biotransformation

Metabolites are identified in this monograph by European Union (EU) reference numbers included in parentheses after the metabolite names.

(a) Oral administration

In a study in which acetochlor was administered by oral intubation to young adult male and female Sprague-Dawley rats, described in section 1.1 above (see also Table 2 in that section), the high-performance liquid chromatographic (HPLC) profiles of radioactivity in pooled urine samples were comparable between sexes and test groups. The urinary metabolites that could be isolated and identified are presented in Table 9, together with their average percentage of dose for each sex.

The isolation and identification of metabolites in faeces were difficult because of the complexity of the faecal metabolite mixture. No individual metabolite was found to be predominant, and the individual metabolites identified were each less than 1% of the dose. The distribution of radioactivity over the different faecal fractions isolated is given in Table 10.

No metabolites could be characterized that were bound to the solid faecal residues. HPLC analysis of the *n*-hexane-soluble fraction revealed the metabolites shown in Table 11.

Table 9. Metabolites identified in urine of rats treated with acetochlor (10 and 400 mg/kg bw)[a]

Metabolite (trivial name[b])	% of dose		HPLC fraction (metabolite no.)	EU reference no.
	Males	Females		
sec-Amide mercapturic acid	5–14	8–23	6 (3)	40
p-Amino phenyl sulfate	2–3.6	2.2–5	3A (4)	37
1-Hydroxyethyl *sec*-amide mercapturic acid	2–3.6	0.7–1.7	3B (7)	38
sec-Amide mercapturic acid sulfoxide	1.2–3.2	1.4–2.4	4 (8)	39
1-Hydroxyethyl *sec*-amide mercapturic acid sulfoxide	0.5–1.8	1.0–1.7	7 (9)	57
Hydroxymethyl *sec*-amide methyl sulfoxide	0.9–2.1	2.7–4.1	8 (11)	41
1-Hydroxyethyl *sec*-amide methyl sulfone	1.1–2.5	1.1–2.7	9B (12)	42
2-Hydroxyethyl *sec*-amide methyl sulfone	0.8–0.9 (only C, D)	0.7–1.4	9C (14)	43
tert-Amide mercapturic acid	–	0.5–1.0	10A (15)	44
Hydroxymethyl *sec*-amide methyl sulfone	0.3–1.0	0.5–1.0	10B (16)	45
sec-Amide methyl sulfoxide	0.5–1.3	2.3–2.7	12 (17)	46
sec-Amide methyl sulfone	0.1 (only C, D)	0.5	14 (18)	10
sec-Amide chloride	0.2–0.4	0.4–0.9	15 (19)	8
sec-Amide methyl sulfide	–	–	15 (20)	36

bw: body weight; EU: European Union; HPLC: high-performance liquid chromatography

[a] Average five rats of each sex. See Table 2 for group descriptions.

[b] "*sec*-amide": *N*-(2-ethyl-6-methylphenyl) acetamide moiety of acetochlor; "*tert*-amide": *N*-ethoxymethyl-*N*-(2-ethyl-6-methylphenyl) acetamide moiety of acetochlor.

Source: Carr et al. (1983).

Table 10. Percentage of dose represented by the faecal fractions isolated[a]

Faecal fraction	% of dose	
	Males	**Females**
Faecal-bound residues	4.9–7.4	4.9–6.4
Hexane-soluble fraction	2.3–6.7	2.6–4.8
Water-soluble fraction	15.9–35.8	10.6–27.4

[a] Average five rats of each sex.
Source: Carr et al. (1983).

Table 11. Faecal metabolites identified in the n-hexane-soluble fraction of pooled faecal samples of rats collected up to 72 hours[a]

Metabolite (trivial name[b])	% of dose		HPLC fraction (metabolite no.)	EU reference no.
	Males	**Females**		
Acetochlor	< 1 (from traces to 6%)	< 1 (from traces to 6%)	3 (1)	1
sec-Amide chloride	< 1	< 1	1 (19)	8
sec-Amide-S-S-*sec*-amide	< 1	< 1	6 (24)	58
sec-Amide-S-S-*tert*-amide	< 1	< 1	5 (25)	47
sec-Amide-S-*tert*-amide	< 1	< 1	4 (27)	48
sec-Amide-S-O-*tert*-amide	< 1	< 1	2 (28)	49
sec-Amide-S-*sec*-amide	< 1	< 1	2 (29)	50

EU: European Union; HPLC: high-performance liquid chromatography
[a] Average five rats of each sex.
[b] "*sec*-amide": *N*-(2-ethyl-6-methylphenyl) acetamide moiety of acetochlor; "*tert*-amide": *N*-ethoxymethyl-*N*-(2-ethyl-6-methylphenyl) acetamide moiety of acetochlor.
Source: Carr et al. (1983)

The faecal metabolite that could be isolated and identified in the *n*-hexane fraction at 12 hours after treatment is *sec*-amide mercapturic acid (EU reference no. 40). However, this metabolite could not be detected in the combined 72-hour faecal sample. The water-soluble fraction was subjected to Raney nickel desulfuration after extraction with ethyl acetate at neutral and acidic pH. The HPLC analysis of the reaction products indicated the presence of thioethers and dithioethers of *sec*-amide, *tert*-amide and 1-hydroxy *sec*-amide metabolites.

Glutathione (GSH) conjugation of acetochlor occurs through displacement of the chlorine atom from the chloromethyl group. The GSH residue is further metabolized to form the corresponding mercapturic acid conjugate. The most abundant metabolite that was isolated and identified in urine is the *sec*-amide mercapturic acid conjugate (5–23% of the dose). This metabolite was detected in faeces at 12 hours after administration. The identification of hydroxylated side-chain and sulfoxide analogues confirmed this route of metabolism. The mercapturic acid conjugate *tert*-amide mercapturic acid (EU reference no. 44) resulting from conjugation of acetochlor with GSH has also been found in urine, but in much lower concentrations (0.5–1.0% of the dose in females only). In the rat, GSH and mercapturic acid conjugates are excreted in the bile and are further metabolized in the gastrointestinal tract by the C–S lyases of the microbial flora. The thiol products that result from this reaction may either be reabsorbed or react with each other to form thioethers and dithioethers, which remain in the gastrointestinal tract and are excreted with the faeces. The identification of thioethers and dithioethers

of *sec*-amides and *tert*-amides confirmed this route of metabolism. The thiols that are formed in the gastrointestinal tract as a result of C–S lyase activity have not been found, but the *S*-methylated derivative (EU reference no. 36) originating from its mercapturic acid analogue (EU reference no. 40) has been identified in urine. This points to enterohepatic circulation of the thiols and further metabolism by *S*-methylation and *S*-oxidation. The identification of the *S*-oxidation products and their hydroxylated side-chain products confirms enterohepatic circulation.

A much less important metabolic pathway is the formation of *N*-dealkylated acetochlor or *sec*-amide chloride (EU reference no. 8). As this metabolite has been identified in urine as well as in faeces, there are two possible routes by which it is formed. The first route involves oxidative *N*-dealkylation of acetochlor. The other route is more complicated and consists of an *O*-dealkylation step of the *N*-ethoxymethyl side-chain followed by glucuronic acid conjugation. The glucuronic acid conjugate is excreted in the bile and deconjugated in the gastrointestinal tract by bacterial glucuronidase activity. This reaction yields the *N*-hydroxymethyl compound, which is unstable and degrades to the *sec*-amide chloride (EU reference no. 8). This metabolite can be reabsorbed from the gastrointestinal tract and further metabolized through GSH conjugation. The last metabolite that was identified is the sulfate conjugate of *p*-amino methylethylphenol (EU reference no. 37). This conjugate is indicative of the formation of a *p*-aminophenol through a combination of aniline *para*-hydroxylation and amide bond cleavage of metabolite precursors.

Only a trace amount of unchanged acetochlor (average < 1% of the administered dose) was found in the faeces. This indicates that most of the radioactivity that has been measured in the faeces originates from biliary excretion.

In summary, acetochlor was rapidly absorbed from the gastrointestinal tract, and the ^{14}C radioactivity was rapidly excreted in the urine and faeces, which contained 76–85% of the dose for males and 76–87% of the dose for females, depending on size and number of doses. The elimination pathways did not appear to be affected by the sex of the animal, and the percentages were essentially the same, despite a 40-fold difference in dose levels. The accumulation of ^{14}C radioactivity in body tissues or organs was very small, which accounted for about 1% of the administered dose. The whole-body elimination takes place in a rapid (α) phase, with a half-life of 5.4–10.4 hours, and a slow late (β) phase, with a half-life of 129–286 hours. The slow elimination phase can be explained by the significant amount of radioactivity that is still bound to the red cell fraction of the blood at 7 days after dosing (2–3% of the administered radioactivity).

The predominant route of metabolism of acetochlor in the rat is the GSH conjugation pathway. Through this pathway, *S*-conjugates are formed, which are excreted in the bile and undergo cleavage of the C–S bond in the gastrointestinal tract. The emerging thiol products are then reabsorbed or condensed to form thioethers and dithioethers, which are excreted in the faeces. The reabsorbed thiols are methylated and further metabolized by *S*-oxidation and side-chain hydroxylation. In addition to the metabolites that are representative of the GSH conjugation pathway, *N*-dealkylation of acetochlor and formation of *p*-amino methylethylphenol (EU reference no. 37) were also identified. From the very low percentage (< 1% of the dose on average) of unchanged acetochlor that could be recovered from the faeces, it can be concluded that acetochlor is very well absorbed from the gastrointestinal tract (Carr et al., 1983).

A study was conducted to investigate the nature of excretory biotransformation products of acetochlor in rats following oral administration of the ^{14}C-labelled compound at nominal doses of 10 and 200 mg/kg bw. The dosing of animals and distribution and excretion of radioactivity have been described in detail above (see section 1.1; Hawkins, Kirkpatrick & Dean, 1988, 1989a,b).

The urine samples collected for 24 hours were pooled for each subgroup of five animals of the same sex. The metabolites were isolated from the urine by means of extraction using C18 Bond Elut sorbent columns. The methanol column eluates were analysed by thin-layer chromatography (TLC) in solvent systems H (chloroform/methanol/acetic acid, 90:10:3) and J (chloroform/methanol/formic acid/water, 70:25:3:3) without further concentration. A fraction of the urine samples was

subjected to incubation with β-glucuronidase/sulfatase before column extraction. The methanol eluates from the urine samples treated with the enzyme mix were analysed only by TLC with solvent system H. The faecal samples collected for 24 hours were pooled for each subgroup of five animals of the same sex. The pooled homogenates were extracted sequentially with ethyl acetate, acetonitrile and acetonitrile/water (7:3). The ethyl acetate extracts were concentrated and analysed by TLC with solvent system H. The acetonitrile extracts (with and without water) were purified by sorbent extraction as described for urine.

The TLC analysis showed that urine contained at least 15 radiolabelled components with a wide range of polarities. No unchanged acetochlor was detected in urine (Table 12). The pretreatment with non-radiolabelled acetochlor did not significantly affect the qualitative or quantitative profile of urinary metabolites, but a quantitative difference was observed when rats were administered higher doses (200 mg/kg bw). All urine samples contained a single major radioactive component (EU reference no. 40) of low mobility in solvent system H (H1; Table 12) and high mobility in solvent system J (J9; Table 13). This component co-chromatographed with the reference compound mercapturic acid conjugate of acetochlor after removal of the ethoxymethyl side-chain from the nitrogen. This component accounted for 22–27% (male) or 28–37% (females) in H-J TLC systems at the dose of 10 mg/kg bw. This metabolite accounted for a lower (9–16%) proportion of the 200 mg/kg bw dose, and this was the main quantitative difference between the urinary profiles at the high and low dose levels. Other metabolites that were identified were *sec*-amide methyl sulfoxide (EU reference no. 46) and *tert*-amide thiolactic acid (EU reference no. 70). The cysteine conjugate of acetochlor, *sec*-amide cysteine (EU reference no. 67), appeared to be a minor metabolite. Comparative chromatography of metabolites before and after incubation with enzymes indicated that glucuronide and sulfate conjugates were relatively minor. The incubation of urine with β-glucuronidase/sulfatase enzymes resulted in a small reduction (19.1–12.8% of the dose) in polar components of the solvent system H, indicating that sulfate and/or glucuronide conjugates were minor components in urine. When urine samples were incubated with enzymes and analysed using solvent system H, component H9 co-chromatographed with the reference compound (EU reference no. 5), which resulted from *N*-dealkylation of acetochlor and retained the chlorine atom. Approximately 80% of the radioactivity in 0- to 24-hour faecal samples was extracted into ethyl acetate and acetonitrile/water. These extracts contained 22% (males) and 16% (females) of the administered dose at 200 mg/kg bw and showed complex patterns of metabolites, none of which was formally characterized. Unchanged acetochlor appeared to be a minor faecal metabolite.

In summary, following oral administration to male and female rats, acetochlor was extensively metabolized, and the metabolites were excreted predominantly in urine. No parent compound was detected in the urine. The major routes of metabolism involved *N*-dealkylation and displacement of the chlorine atom from the chloromethyl group by GSH. The GSH conjugate was found to be further degraded to mercapturic acid and thiolactic acid conjugates. The presence of *sec*-amide methyl sulfoxide (EU reference no. 46) in the urine is indicative of biliary excretion of GSH conjugates and their degradation products (cysteine and mercapturic acid conjugates), cleavage of the C–S bond by β-lyase of the gut microflora and *S*-methylation after reabsorption of the thiols from the gastrointestinal tract. The metabolite profiles were similar after administration of ^{14}C-labelled acetochlor at 10 or 200 mg/kg bw and were not affected by pretreatment of the rats with non-radiolabelled acetochlor prior to administration of the radioactive dose (Hawkins, Kirkpatrick & Dean, 1989c).

The biotransformation of acetochlor was investigated in a study in which 12 male and six female Sprague-Dawley rats were administered a single oral dose of ^{14}C-phenyl-labelled acetochlor at 10 or 200 mg/kg bw in polyethylene glycol 600 and the bile ducts of four of the male rats (two each at 10 and 200 mg/kg bw) were cannulated 24 hours prior to dosing. This study (Jones, 1990) was described in section 1.1 above (see also Table 6).

Table 12. Quantification of acetochlor metabolites expressed as percentages of a single oral dose (10 or 200 mg/kg bw) or a single oral ^{14}C dose (10 mg/kg bw) after 14 unlabelled doses (10 mg/kg bw) to rats, analysed with solvent system H

Metabolite (trivial name[a])	% of administered dose						TLC fraction	EU reference no.
	10 mg/kg bw		200 mg/kg bw		10 mg/kg bw (14 + 1 days)			
	M	F	M	F	M	F		
	21.1	19.3	16.2	15.8	20.2	21.9	Polar fraction	
sec-Amide mercapturic acid	22.1	29.8	8.9	10.5	22.1	28.3	H1	40
tert-Amide thiolactic acid	4.4	3.0	4.2	2.2	4.0	2.7	H2	70
	2.1	2.6	1.3	2.0	1.8	2.3	H3/H4	
	4.9	4.7	6.2	6.0	4.4	4.4	H5	
sec-Amide methyl sulfoxide	3.5	5.0	4.3	12.0	3.4	5.4	H6	46
			1.1	2.6			H7	
	0.4	1.0	0.4		0.7	1.0	H8	
	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	H9/H10	

bw: body weight; EU: European Union; F: female; M: male; TLC: thin-layer chromatography
[a] "*sec*-amide": *N*-(2-ethyl-6-methylphenyl) acetamide moiety of acetochlor; "*tert*-amide": *N*-ethoxymethyl-*N*-(2-ethyl-6-methylphenyl) acetamide moiety of acetochlor.
Source: Hawkins, Kirkpatrick & Dean (1989c)

Table 13. Quantification of acetochlor metabolites expressed as percentages of a single oral dose (10 or 200 mg/kg bw) or a single oral ^{14}C dose (10 mg/kg bw) after 14 unlabelled doses (10 mg/kg bw) to rats, analysed with solvent system J

Metabolite (trivial name[a])	% of administered dose						TLC fraction	EU reference no.
	10 mg/kg bw		200 mg/kg bw		10 mg/kg bw (14 + 1 days)			
	M	F	M	F	M	F		
	1.5	0.8	1.3	1.0	0.9	0.6	Polar fraction	
	6.9	6.1	5.2	7.3	5.0	4.6	J1	
sec-Amide cysteine	4.5	3.0	4.0	2.8	3.8	6.3	J2–J4	67
	3.7	4.1	2.8	3.2	2.7		J5/6	
	6.1	5.9	3.3	1.9	6.9	4.8	J7	
	3.7	3.5	3.4	2.0	3.5	4.8	J8	
sec-Amide mercapturic acid	24.8	32.3	12.1	15.9	27.3	37.2	J9	40
	3.9	3.5	5.6	4.1	3.4	2.5	J10	
sec-Amide methyl sulfoxide, *tert*-amide thiolactic acid	2.8	4.5	3.2	10.8	2.1	4.1	J11	46, 70
	0.6	1.2	1.2	1.8	0.8		J12	
	0.2	0.3	0.5	0.5	0.8	1.0	J13	

bw: body weight; EU: European Union; F: female; M: male; TLC: thin-layer chromatography
[a] "*sec*-amide": *N*-(2-ethyl-6-methylphenyl) acetamide moiety of acetochlor; "*tert*-amide": *N*-ethoxymethyl-*N*-(2-ethyl-6-methylphenyl) acetamide moiety of acetochlor.
Source: Hawkins, Kirkpatrick & Dean (1989c)

Metabolites were isolated from urine and bile samples by solid sorbent extraction with Bond Elut C-8 columns using dichloromethane, ethyl acetate and ethanol as eluents. Only dichloromethane was used for the elution of bile metabolites following incubation with β-glucuronidase to hydrolyse glucuronic acid conjugates.

Metabolites were isolated from faeces by extraction of the homogenates with ethyl acetate, acetonitrile and acetonitrile/water (1:1). The combined acetonitrile fractions were evaporated and the metabolites extracted with Bond Elut C-8 columns using methanol as the eluent.

The quantification and characterization of the metabolite fractions are shown in Table 14 for urine and Table 15 for bile.

Table 14. Quantification of characterized acetochlor metabolites in urine of non-bile duct–cannulated rats expressed as percentages of the administered radioactivity after a single oral dose

	% of administered dose					
	10 mg/kg bw		200 mg/kg bw			EU reference no.
Metabolite (trivial name[a])	M	F	M	F	Fraction	
sec-Amide mercapturic acid	24.8	32.3	12.1	15.9	U9	40
sec-Amide methyl sulfoxide	< 2.8	< 4.3	< 3.2	< 10.8	U11	46

bw: body weight; EU: European Union; F: female; M; male

[a] "*sec*-amide": *N*-(2-ethyl-6-methylphenyl) acetamide moiety of acetochlor; "*tert*-amide": *N*-ethoxymethyl-*N*-(2-ethyl-6-methylphenyl) acetamide moiety of acetochlor.

Source: Jones (1990)

Table 15. Quantification of characterized acetochlor metabolites in bile of male bile duct–cannulated rats expressed as percentages of the administered radioactivity after a single oral dose

	% of administered dose					
	10 mg/kg bw		200 mg/kg bw			EU reference no.
Metabolite (trivial name[a])	M	F	M	F	Fraction	
sec-Amide glutathione	3.8	–	3.8	–	B5	51
tert-Amide glutathione	8.9	–	13.8	–	B7	54
O-dealkylated acetochlor *O*-glucuronide	30.2	–	41.1	–	B9	52
tert-Amide mercapturic acid	5.7	–	3.6	–	B15	44

bw: body weight; EU: European Union; F: female; M; male

[a] "*sec*-amide": *N*-(2-ethyl-6-methylphenyl) acetamide moiety of acetochlor; "*tert*-amide": *N*-ethoxymethyl-*N*-(2-ethyl-6-methylphenyl) acetamide moiety of acetochlor.

Source: Jones (1990)

No qualitative sex differences in metabolite profiles were apparent, although differences were apparent in the relative proportions of the metabolites. In non-bile duct–cannulated rats, the major urinary metabolite (U9) accounted for approximately 25% and 32% of a 10 mg/kg bw dose and 12% and 16% of a 200 mg/kg bw dose for males and females, respectively. The remaining urinary radioactivity was resolved into at least 11 minor metabolites. Urine collected from bile duct–cannulated rats contained at least five metabolites, none of which corresponded to the major metabolite found in non-bile duct–cannulated rats. The cannulated rats were examined for bile metabolite profile. There were no marked differences when the rats were dosed with 10 or 200 mg/kg bw. Two major metabolites were identified: B9, which accounted for 30.2% and 41.1% of the dose at 10 and 200 mg/kg bw, respectively; and B7, representing about 8.9% and 13.8% of the dose at 10 and 200 mg/kg bw, respectively. The remaining 10–13 components represented less than 5% of the dose.

Acetochlor was both rapidly and extensively absorbed following a single oral dose of either 10 or 200 mg/kg bw. This was clearly shown in the excretion data for bile duct–cannulated rats, as 30% of the administered dose was eliminated within 4 hours, and more than 84% of a 10 mg/kg bw dose was eliminated via bile within 24 hours. No marked dose level effect was apparent, as more than 80% of a 200 mg/kg bw dose was eliminated via bile. The small amounts of dose excreted in faeces by bile duct–cannulated rats showed that an oral dose of acetochlor was almost quantitatively absorbed. Hence, the majority of faecal radioactivity in non-cannulated rats was of biliary origin.

As non-bile duct–cannulated rats excreted more than 70% of a 10 mg/kg bw oral dose in urine, the elimination of approximately 85% of a similar dose via bile shows that enterohepatic recirculation of acetochlor metabolites was extensive. The absence of the major urinary metabolite (U9) in the urine of bile duct–cannulated rats also indicates that this metabolite was derived via enterohepatic recirculation. The identification of intermediary metabolites in bile showed that the initial metabolism of acetochlor was principally focused on two reaction sites, the ethoxymethyl side-chain and the chlorine atom. The identification of the major biliary metabolite (B9) as the glucuronide conjugate of *O*-dealkylated acetochlor indicates that this was a preferred biotransformation reaction. Conjugation of acetochlor with GSH via displacement of the chlorine atom was the second preferred biotransformation reaction. In addition to the GSH conjugate of acetochlor (B7), bile also contained the corresponding mercapturic acid (B15). A second minor metabolite was identified as the GSH conjugate of *N*-dealkylated acetochlor (B5). No acetochlor was detected in the bile in rats administered 200 mg/kg bw; therefore, low faecal excretion in bile duct–cannulated rats appears to represent the unabsorbed dose.

In summary, an oral acetochlor dose of 10 or 200 mg/kg bw was rapidly and almost completely absorbed by the rat and was subject to extensive metabolism. The two main biotransformation pathways involved *O*-dealkylation and glucuronidation of the *N*-ethoxymethyl side-chain or the complete cleavage of this moiety and displacement of the chlorine atom from the chloromethyl group by GSH, leading to mercapturic acid formation. The metabolic intermediates were extensively eliminated via bile, with evidence of enterohepatic circulation (Jones, 1990).

Urine samples collected from male and female rats during the first day after a single oral dose of ^{14}C-labelled acetochlor at 10 or 200 mg/kg bw were used to further characterize metabolites by TLC, liquid chromatography, HPLC and gas–liquid chromatography following derivatization. The urine used for isolation and identification of metabolites reported here was obtained from the Hawkins, Kirkpatrick & Dean (1988, 1989a) studies.

Three additional minor urinary metabolites of acetochlor were characterized by gas chromatography–mass spectromety. Their relative proportions were determined by TLC using a linear analyser and are expressed as percentage of the dose in Table 16.

Table 16. Quantification of additional acetochlor metabolites in the urine of rats expressed as percentages of the administered radioactivity after a single oral dose of 10 or 200 mg/kg bw

	% of the dose					
	10 mg/kg bw		200 mg/kg bw		TLC fraction	EU reference no.
Metabolite (trivial name[a])	M	F	M	F		
p-OH-EMA	3.7	3.5	3.4	2.0	U8	71
sec-Amide chloride	0.6	1.2	1.2	1.8	U12a	8
sec-Amide methyl sulfide					U12b	36

bw: body weight; *p*-OH-EMA: *para*-hydroxy-2-ethyl-6-methylaniline; EU: European Union; F: female; M: male; TLC: thin-layer chromatography

[a] "*sec*-amide": *N*-(2-ethyl-6-methylphenyl) acetamide moiety of acetochlor.

Source: Prout & Gledhill (1991)

para-Hydroxy-2-ethyl-6-methylaniline (*p*-OH-EMA) (EU reference no. 71) corresponds to TLC fraction J8, and *sec*-amide chloride (EU reference no. 8) and *sec*-amide methyl sulfide (EU reference no. 36) both correspond to TLC fraction J12 (Hawkins, Kirkpatrick & Dean, 1989c).

In summary, three additional minor urinary metabolites of acetochlor in the rat were identified in this study: *p*-OH-EMA (EU reference no. 71), *sec*-amide chloride (EU reference no. 8) and *sec*-amide methyl sulfide (EU reference no. 36). The last metabolite confirms the formation of GSH conjugates and derived products that are excreted in the bile and subsequently reabsorbed from the gastrointestinal tract after cleavage of the C–S bond. The *sec*-amide chloride (EU reference no. 8) is indicative of the *N*-dealkylation pathway, whereas *p*-OH-EMA (EU reference no. 71) is the result of *N*-dealkylation followed by amide hydrolysis and *para*-hydroxylation (Prout & Gledhill, 1991).

In another study, the urinary and plasma metabolite profiles were compared in CD rats and CD-1 mice given single oral doses of ^{14}C-labelled acetochlor. In addition, the dose-dependent metabolism of acetochlor was studied over a wide range of dose levels (10–2000 mg/kg bw). The study design is shown in Table 17.

Table 17. Design of comparative metabolism study in rats and mice

Group	No. of animals	Dose (mg/kg bw)	Dose regimen	Sampling times (h)	Samples for radioactivity determination
1	5 rats 6 mice	200	Gavage (corn oil)	24, 48	Urine, faeces
2	4 rats 10 mice	200	Gavage (PEG 600)	17	Blood (cardiac puncture)
3	8 rats	200	6 months of treatment at 1 750 ppm non-labelled acetochlor in diet followed by single gavage dose of ^{14}C-labelled acetochlor at 200 mg/kg bw 24 h prior to termination	24 h after dosing with ^{14}C-labelled acetochlor	Blood (cardiac puncture)
4	5 rats	10, 200, 1 000, 2 000	Gavage (corn oil)	24	Urine

bw: body weight; PEG: polyethylene glycol; ppm: parts per million
Source: Green et al. (1998)

Urinary metabolites in the rat were found to be derived principally from GSH conjugation at the chloramide chlorine atom, the major metabolite being the mercapturic acid (metabolite F; Table 18). In contrast, the metabolite profile in mouse urine was markedly different from that in rat urine. The major metabolite (metabolite H), which still retained the chlorine atom of the chloramide group, was identified as a glucuronide conjugate of the *N*-methylol group remaining after oxidative *O*-de-ethylation of the *N*-ethoxymethyl side-chain. Glucuronides formed from ring side-chain oxidations were also present. The marked difference between the metabolism of acetochlor in rats and mice was further highlighted by treatment of the urine samples with β-glucuronidase. Whereas this enzyme had little effect on the metabolites in rat urine, the retention times of almost all of the mouse metabolites changed after treatment, demonstrating that these were, in fact, glucuronides.

Acetochlor sulfoxide (*sec*-amide methyl sulfoxide; EU reference no. 46) was the major metabolite in plasma from rats given a single oral acetochlor dose of 200 mg/kg bw. This was also the major plasma metabolite in rats treated for 6 months with acetochlor at 1750 parts per million (ppm) in the diet. A second significant metabolite, which was coincident with the chloramide, was present.

Table 18. Metabolites identified in 0- to 24-hour urine of rats and mice treated with a single oral dose of ^{14}C-labelled acetochlor at 200 mg/kg bw

Metabolite (trivial name[a])	HPLC fraction	EU reference no.
Rats		
Hydroxylated *sec*-amide methyl sulfoxide	A	65
Hydroxylated *sec*-amide methyl sulfone	B+C	66
sec-Amide cysteine	D	67
sec-Amide methyl sulfoxide	E	46
sec-Amide mercapturic acid	F	40
sec-Amide methyl sulfone	G	10
sec-Amide chloride	I	8
Mice		
O-Dealkylated acetochlor *O*-glucuronide	H	52
sec-Amide chloride	I	8

bw: body weight; EU: European Union; HPLC: high-performance liquid chromatography
[a] "*sec*-amide": *N*-(2-ethyl-6-methylphenyl) acetamide moiety of acetochlor.
Source: Green et al. (1998)

Mouse plasma, in contrast, contained very little acetochlor sulfoxide (EU reference no. 46), the major metabolite being coincident with the chloramide (*sec*-amide chloride; EU reference no. 8). Both the rate of excretion and the metabolism of acetochlor in the rat differed with dose. The rat eliminated almost all radioactivity within 24 hours when the animals were administered low doses (100–200 mg/kg bw) of acetochlor. However, the time of elimination increased to 48 hours when higher doses (1000–2000 mg/kg bw) were administered. The major metabolite over the entire dose range was the *sec*-amide mercapturic acid (EU reference no. 40). However, as the dose level increased, there was a significant increase in the excretion of glucuronic acid conjugates, which is indicative of a saturation of the GSH conjugation pathway.

In summary, the metabolism of acetochlor differed between rats and mice. In rats, acetochlor was eliminated as urinary metabolites derived from GSH conjugates, whereas in mice, it was eliminated as urinary metabolites derived from glucuronide conjugates. Plasma analysed after 17 hours of acetochlor administration confirmed the difference. The major metabolite in plasma of rats was the *sec*-amide methyl sulfoxide (EU reference no. 46). In contrast, the levels of the *sec*-amide methyl sulfoxide (EU reference no. 46) in mouse plasma were extremely low. Significant levels of the *sec*-amide chloride (EU reference no. 8) were found in the plasma of both the rat and mouse. When the dose of acetochlor to rats was increased, the glucuronide conjugates eliminated by urine increased at the same time, indicating a saturation of the GSH conjugation system; therefore, cells would remain unprotected from other free radicals. Rats eliminated almost all acetochlor within 24 hours when the animals were dosed with low levels of acetochlor (10–200 mg/kg bw); however, higher doses (1000–2000 mg/kg bw) were eliminated within 48 hours (Green et al., 1998).

A study was conducted to determine the nature of the acetochlor metabolites excreted in rat bile, the extent of biliary excretion and the relationship of the bile metabolites to the metabolites previously identified in the urine and faeces. Sprague-Dawley rats were dosed with an approximately 1:1 mixture of acetochlor labelled with phenyl-^{14}C and with ^{13}C in the carbon 2 position through different routes, but the dose level was 7 mg/kg bw for all dose groups. The study design is shown in Table 19.

Table 19. Design of study to determine acetochlor metabolites in rat bile

Group	No. of rats of each sex	Dose (mg/kg bw)	Route of administration	Primary samples collected and sampling interval
1	3	7	Intravenous	Bile was collected at 30 min intervals for 6 h
2	2	7	Intragastric injection	Bile was collected at 30 min intervals for 7.5 h
3	3	7	Oral gavage	Bile, urine, faeces; bile was collected at 3, 6, 12, 24, 48 and 72 h
4	2	7	Intravenous	Bile duct cannula of donor rat was inserted into duodenum of acceptor rat; samples were collected by bile duct cannulation of acceptor rat; bile was collected at 30 min intervals for 7 h

bw: body weight
Source: Yalamanchili, Oppenhuizen & Wilson (1985, 1987)

Table 20. Mean excretion of radioactivity in the bile of rats of test groups 1, 2, 3 and 4

	% of administered radioactivity excreted in bile	
Group	**Males**	**Females**
1[a]	60	52
2[b]	7	8
3[c]	72	65
4[d]	17	15

[a] Amount recovered within 6 hours of intravenous dose.
[b] Amount recovered within 7.5 hours of intragastric injection.
[c] Amount recovered within 72 hours of oral gavage.
[d] Amount recovered from recipient rats within 7 hours after intravenous dosing of donor rats.
Source: Yalamanchili, Oppenhuizen & Wilson (1985, 1987)

The excretion of radioactivity in the bile is shown in Table 20. More than 50% of the administered dose was recovered only from those bile duct–cannulated rats administered acetochlor orally and intravenously. The rate of excretion of total radioactivity in the bile of the rats of group 1 followed a biphasic pattern, with half-lives of 0.43 and 0.31 hour for the initial phase (α phase) for males and females, respectively, and half-lives of 2.1 and 2.2 hours for the late phase (β phase) for males and females, respectively.

Preparative HPLC with radioactivity detection separation of the bile samples from the four test groups revealed eight major metabolites. The percentage of identified acetochlor metabolites in the pooled bile samples collected from the four test groups is given in Table 21.

There were no important differences in radioactivity HPLC profiles between the pooled bile samples from male and female animals within the experimental groups. The biliary metabolites that were isolated and identified are presented in Table 22, together with their range of percentage of dose for each sex.

The GSH, cysteinylglycine and cysteine conjugates were not detected in the bile of rats from group 4. This is the group where the bile duct cannula of each donor rat (receiving an intravenous dose) was inserted into the duodenum of an acceptor rat from which the bile was collected for analysis. The most abundant metabolite present in the bile of acceptor rats was *tert*-amide mercapturic acid (EU reference no. 44). This metabolite represented 49.1% and 44.7% of the radioactivity excreted in the bile of male and female animals, respectively. Both the glucuronic acid and GSH conjugates and their metabolites could be detected at 30 minutes after administration of ^{14}C-labelled acetochlor.

Table 21. Percentage of radioactivity excreted in the bile of male and female rats from all test groups representative of the eight major metabolites identified

	% of radioactivity excreted in the bile	
Group	**Males**	**Females**
1	86.6	76.5
2	84.4	77.5
3	75.9	76.3
4	77.0	74.5

Source: Yalamanchili, Oppenhuizen & Wilson (1985, 1987)

Table 22. Metabolites and their percentage of dose range isolated from the bile samples of male and female rats from all test groups

	% of dose			
Metabolite (trivial name[a])	**Males**	**Females**	**HPLC fraction (metabolite no.)**	**EU reference no.**
sec-Amide glutathione	0.1–3.0	0.1–3.7	A (1)	51
sec-Amide mercapturic acid	0.3–3.7	0.4–3.2	B (2)	40
O-Dealkylated acetochlor *O*-glucuronide	1.1–26.8	1.6–33.3	C (3)	52
Hydroxymethyl acetochlor *O*-glucuronide	0.4–2.5	0.1–1.5	D (4)	53
tert-Amide glutathione	0.6–9.3[b]	0.3–9.4[b]	E (5)	54
tert-Amide mercapturic acid	0.8–15.1	0.9–7.8	F (6)	44
tert-Amide cysteinylglycine	0.6–3.8[b]	0.6–4.4[b]	G (7)	55
tert-Amide cysteine	0.2–1.4[b]	0.4–3.4[b]	H (8)	56

EU: European Union; HPLC: high-performance liquid chromatography

[a] "*sec*-amide": *N*-(2-ethyl-6-methylphenyl) acetamide moiety of acetochlor; "*tert*-amide": *N*-ethoxymethyl-*N*-(2-ethyl-6-methylphenyl) acetamide moiety of acetochlor.

[b] Metabolite was not found in group 4.

Source: Yalamanchili, Oppenhuizen & Wilson (1985, 1987)

Eight major metabolites were identified in groups 1, 2 and 3, and five in group 4. The most abundant metabolite present in the bile of groups 1, 2 and 3 was the *O*-glucuronic acid conjugate of *O*-dealkylated acetochlor (EU reference no. 52). This metabolite is formed by the *O*-dealkylation of the *N*-ethoxymethyl side-chain of acetochlor, yielding the corresponding hydroxymethylene (-N-CH_2-OH) intermediate, which is then conjugated with glucuronic acid. Once this metabolite is excreted in the gastrointestinal tract via the bile, it can be deconjugated by the β-glucuronidase activity of the gut microflora. The hydroxymethylene compound (methylol) is then released again and degrades to *sec*-amide chloride (EU reference no. 8). This gut metabolite can be readily reabsorbed and undergo further liver metabolism. Another glucuronic acid conjugate that was identified is hydroxymethyl acetochlor *O*-glucuronide (EU reference no. 53). Both metabolites belong to the oxidative and glucuronic acid conjugation pathways of the liver.

All the other metabolites are part of the GSH conjugation pathway of the liver. Acetochlor and its *N*-dealkylated metabolite are conjugated with GSH through displacement of the chlorine atom from the chloromethyl group (EU reference nos 51 and 54). The GSH residue is then gradually degraded by gamma-glutamyltranspeptidase (GGT) (formation of cysteinylglycine conjugate, EU reference no. 55), cysteinylglycinase (formation of cysteine conjugate, EU reference no. 56) and *N*-acetyltransferase (formation of mercapturic acid conjugates, EU reference nos 40 and 44). The

GSH conjugates, the mercapturic acid conjugates and all the intermediates are excreted in the gastrointestinal tract via the bile, where the C–S bond is cleaved by C–S lyase (β-lyase) activity of the gut microflora. The thiol compounds can then be reabsorbed and further metabolized by the liver. The most abundant metabolite in the bile of rats from group 4 was *tert*-amide mercapturic acid (EU reference no. 44); the intermediate metabolites of that pathway could not be isolated and characterized.

In summary, a substantial amount of the administered radioactivity is excreted in the bile after 6 hours (60% in males, 52% in females) by the intravenous route and 72 hours (72% in males, 65% in females) by the oral route. There were no sex-related differences in excretion in the bile. Eight metabolites were identified in the bile of rats treated with intravenous, intragastric or single oral gavage doses. Five major metabolites were identified in bile duct–cannulated donor and recipient rats. From the results of the test with the bile duct–cannulated donor and recipient rats, it can be concluded that the metabolites that are excreted in the bile undergo enterohepatic circulation and are partly excreted again in the bile (14–20% of administered radioactivity) after further metabolism in the liver. On the basis of the metabolites that were identified in the bile in this study, it can be concluded that there are two main metabolic pathways leading to biliary excretion in the rat: the glucuronic acid conjugation pathway and the GSH conjugation pathway (Yalamanchili, Oppenhuizen & Wilson, 1985).

(b) Other routes of administration

Urine samples collected during a dermal penetration study of acetochlor with monkeys (Maibach, 1983) were used to identify the major metabolites of acetochlor. Rhesus monkeys were dosed with ^{14}C-labelled acetochlor intravenously, intramuscularly, subcutaneously or topically. For each dose group, the urine samples for each monkey containing the maximum level of ^{14}C radioactivity were prepared by concentration and/or Sep-Pak treatment for a chromatographic evaluation of the urine-contained ^{14}C radioactivity.

Two mercapturic acid metabolites were found to predominate in the histograms generated by the HPLC/liquid scintillation counting analyses in each dose group. These metabolites together accounted for 36–52% of the ^{14}C radioactivity that was profiled. The major metabolite (23–38% of the ^{14}C radioactivity in the samples analysed) was identified by HPLC retention time and mass spectral analysis as 3-[*N*-ethoxymethyl-*N*-(2-ethyl-6-methylphenyl)acetamido]thio-2-acetylaminopropanoic acid. It is also known as *tert*-amide mercapturic acid conjugate (EU reference no. 44). The second most abundant metabolite (13–16% of the HPLC radioactivity in the samples analysed) was identified by HPLC retention time and mass spectral analysis as 3-[*N*-(2-ethyl-6-methylphenyl)acetamido]thio-2-acetylaminopropanoic acid (*sec*-amide mercapturic acid conjugate; EU reference no. 40). No unchanged acetochlor was found in the urine of any monkey from any dose group, and no other metabolites were found to be present in appreciable quantities relative to the two mercapturic acids.

The metabolic fate of acetochlor in rhesus monkey urine was not significantly altered by the different routes or forms of administration and was very consistent from animal to animal. The rate of metabolite elimination was rapid following the intravenous and intramuscular administrations and was somewhat slower following the subcutaneous and topical applications. However, the distribution of metabolic end-products indicated that the mercapturate conjugates were consistently formed and eliminated and accounted for the majority of the ^{14}C radioactivity in the urine.

In summary, the two predominant metabolites of acetochlor in rhesus monkey urine were mercapturic acid conjugates. The two metabolites were identified to be the mercapturic acid conjugates of acetochlor, *tert*-amide mercapturic acid (EU reference no. 44) and *sec*-amide mercapturic acid (EU reference no. 40). From the time course analysis of urine samples collected from the monkeys treated dermally, it appears that the *tert*-amide mercapturic acid is excreted initially, followed by the *sec*-amide mercapturic acid. The metabolism data obtained in this study demonstrate that the GSH conjugation pathway leading to the formation of mercapturic acid conjugates is by far the predominant route of metabolism of acetochlor in the rhesus monkey (Livingston, 1983).

A study was conducted to characterize acetochlor metabolites in urine following intravenous administration to rhesus monkeys at two different doses (also described in section 1.1 above). In this study, two groups of three male rhesus monkeys were administered ^{14}C-phenyl-labelled acetochlor intravenously, using propylene glycol/ethanol/saline (60:10:30) as the dose vehicle, at a dose level of 0.005 or 0.05 mg/kg bw.

The metabolism of acetochlor was very similar for both groups, as indicated by comparisons of their metabolite profiles in urine. The major metabolites, each of which constituted more than 10% of the metabolite profile for all six monkeys, were the *tert*-amide mercapturic acid (EU reference no. 44) and the *O*-glucuronic acid conjugate of *O*-dealkylated acetochlor (EU reference no. 52). These two metabolites constituted 19.93–31.58% and 12.72–22.76% of the urine metabolite profiles, respectively. No other metabolite peaks exceeded 10% of the metabolite profile. Only one other identified metabolite, the *sec*-amide mercapturic acid (EU reference no. 40), exceeded 5% of the profile for any monkey (Table 23).

Table 23. Average percentage of radioactivity in chromatographic profiles of the three major metabolites identified in the urine of monkeys of each dose group

		Average % of profile (*n* = 3)		
Dose level (mg/kg bw)	**Collection period (h)**	***tert*-Amide mercapturic acid (EU reference no. 44)**	**Glucuronide of acetochlor (EU reference no. 52)**	***sec*-Amide mercapturic acid (EU reference no. 40)**
0.005	0–48	27.26	17.46	6.08
0.05	0–48	25.39	19.15	5.30

bw: body weight; EU: European Union
Source: Kurtzweil (2014)

Two other metabolites, the *sec*-amide chloride (EU reference no. 8) and the *tert*-thioacetic acid (EU reference no. 4), with maximum percentages of profile values of 3.89% and 3.46%, respectively, were also identified. Unchanged acetochlor was not observed in the urine from any of the monkeys. Profiling of metabolites in the faeces was not conducted, because the percentage of the dose recovered was relatively low.

Three other polar peaks eluting at retention times much earlier than those of the identified metabolites, which reached maxima of 6.47% (P1), 6.85% (P2) and 3.77% (P3) of the profiles, were further characterized using acid pressure hydrolysis. The metabolite isolated from peak P1 was characterized as being hydroxylated at the 1-position of the ethyl group attached to the phenyl ring. Peak P2 consisted of at least two metabolites, one of which was consistent with hydroxylation at the 1-position of the ethyl group attached to the phenyl ring. The other metabolite gave two products upon hydrolysis. One product was consistent with a metabolite with hydroxylation at the methyl group attached to the phenyl ring, whereas the retention time of the other product did not correspond to any of the reference standards. Peak P3 also appeared to consist of at least two metabolites. Acid pressure hydrolysis of each isolated peak gave a metabolite with hydroxylation at the methyl group attached to the phenyl ring and a product with a retention time that did not correspond to any of the reference standards.

Acid pressure hydrolysis of metabolites in urine from monkeys in the low-dose group (1M) gave levels of 2-ethyl-6-methylaniline (EMA; EU reference no. 34) ranging from 65.1% to 68.8% of the mixture. By comparison, EMA levels from monkeys in the high-dose group (2M) ranged from 59.5% to 70.2%. The lowest conversion level was observed for monkey 2M1 of the high-dose group, which had the highest level of polar metabolites. In addition, the levels of EMA obtained from acid hydrolysis were 11–14% higher than the summed percentage distributions for the five metabolites

shown in Table 23 from the urine of a particular monkey. This indicates that the conversion of metabolites to EMA primarily accounts for the five identified metabolites.

In summary, acetochlor was extensively metabolized in the rhesus monkey primarily by two metabolic pathways involving either GSH conjugation or cytochrome P450 (CYP) oxidation followed by glucuronic acid conjugation (Kurtzweil, 2014).

The major metabolic pathways of acetochlor in the rat, mouse and rhesus monkey are illustrated in Figs 2, 3 and 4, respectively.

2. Toxicological studies

2.1 Acute toxicity

The results of acute toxicity studies with acetochlor (including skin and eye irritation and dermal sensitization studies) are summarized in Table 24.

(a) Oral administration

Rats

In an acute oral toxicity study, groups of five Sprague-Dawley rats of each sex received a single oral dose of undiluted acetochlor (purity 96.3%) at 1600, 2172, 2947 or 4000 mg/kg bw.

The incidences of mortality were as follows: 1600 mg/kg bw: 0/5 males and 2/5 females; 2172 mg/kg bw: 2/5 males and 2/5 females; 2947 mg/kg bw: 4/5 males and 5/5 females; and 4000 mg/kg bw: 5/5 males and 5/5 females. All deaths occurred within 2 days after administration. Signs of toxicity included salivation, lethargy, ptosis, perioral wetness and red discoloration around the mouth and fur. Signs of systemic toxicity included ataxia, tremors and convulsions; 5/6 rats with at least one of these clinical signs died. Diarrhoea and the presence of reddish material in the urine occurred sporadically. Most clinical signs of toxicity were observed on the day of dosing and/or the following day. At necropsy, gastrointestinal inflammation, presence of red-brown material in the intestines and discoloured hepatic tissue were observed.

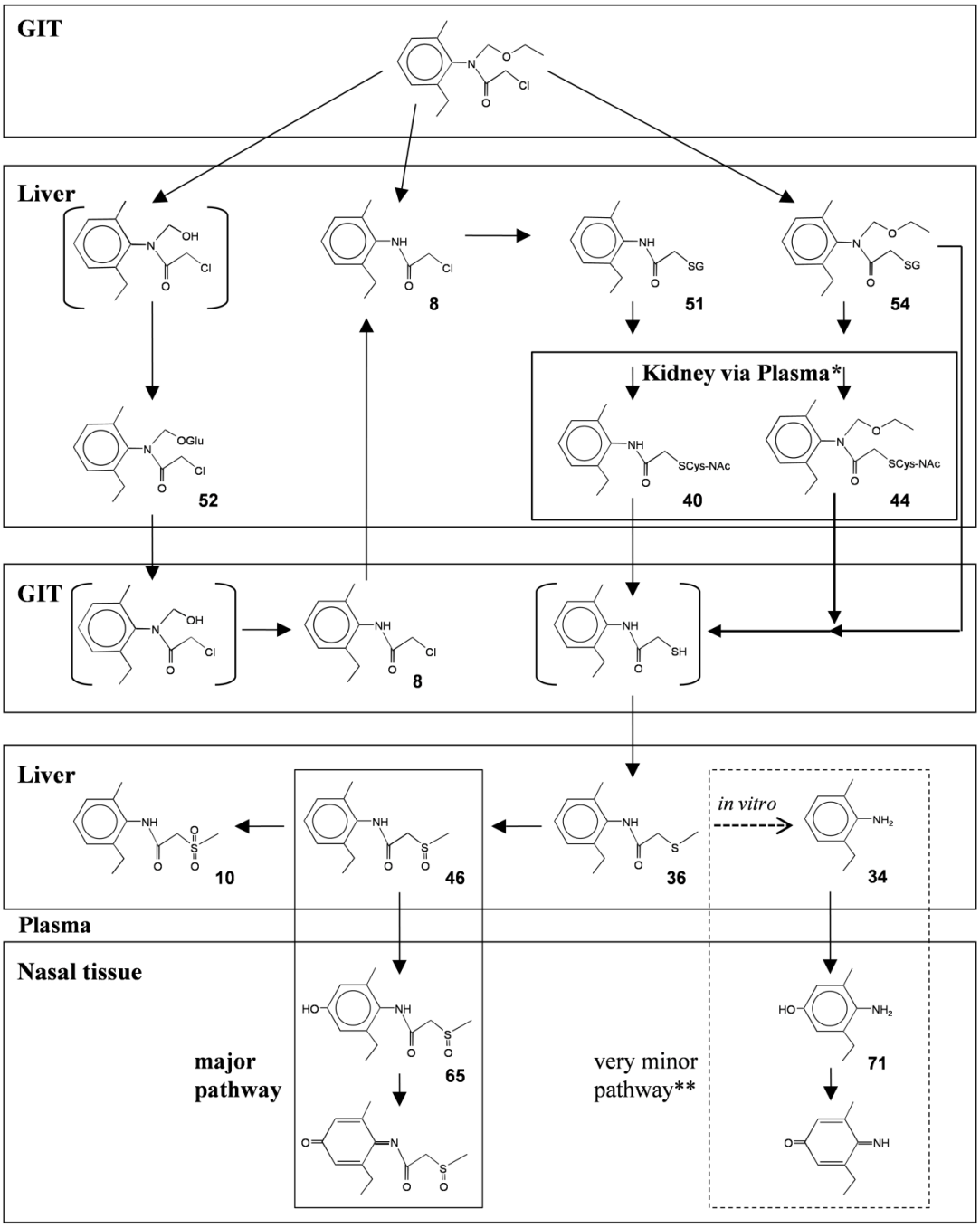
Under the study conditions utilized, the acute oral median lethal dose (LD_{50}) values for acetochlor were 2389 mg/kg bw for males, 1929 mg/kg bw for females and 2148 mg/kg bw for both sexes combined (Branch, 1982b).

In a second acute oral toxicity study, acetochlor (purity 90.5%) suspended in corn oil was administered as a single dose at a constant dose volume of 10 mL/kg bw to groups of five male and five female CD (Sprague-Dawley origin) rats at a dose of 2324, 3000, 3873 or 5000 mg/kg bw.

The incidences of mortality were as follows: 2324 mg/kg bw: 0/5 males and 0/5 females; 3000 mg/kg bw: 1/5 males and 1/5 females; 3873 mg/kg bw: 1/5 males and 2/5 females; and 5000 mg/kg bw: 4/5 males and 4/5 females. All deaths occurred within 6 days of administration. Clinical signs included decreased motor activity, lethargy, piloerection, diarrhoea, hunched posture, salivation and ungroomed appearance. Rats in the lowest-dose group recovered by day 2. At higher dose levels, recovery was not complete until day 12. Necropsy of decedents did not indicate a clear organ-specific toxicological effect. Necropsy of surviving animals on day 15 was unremarkable.

Under the study conditions utilized, the acute oral LD_{50} values for acetochlor were 4238 mg/kg bw for males and 4015 mg/kg bw for females (Cummins, 1986b).

Fig. 2. Major metabolic pathway of acetochlor in the rat[a]



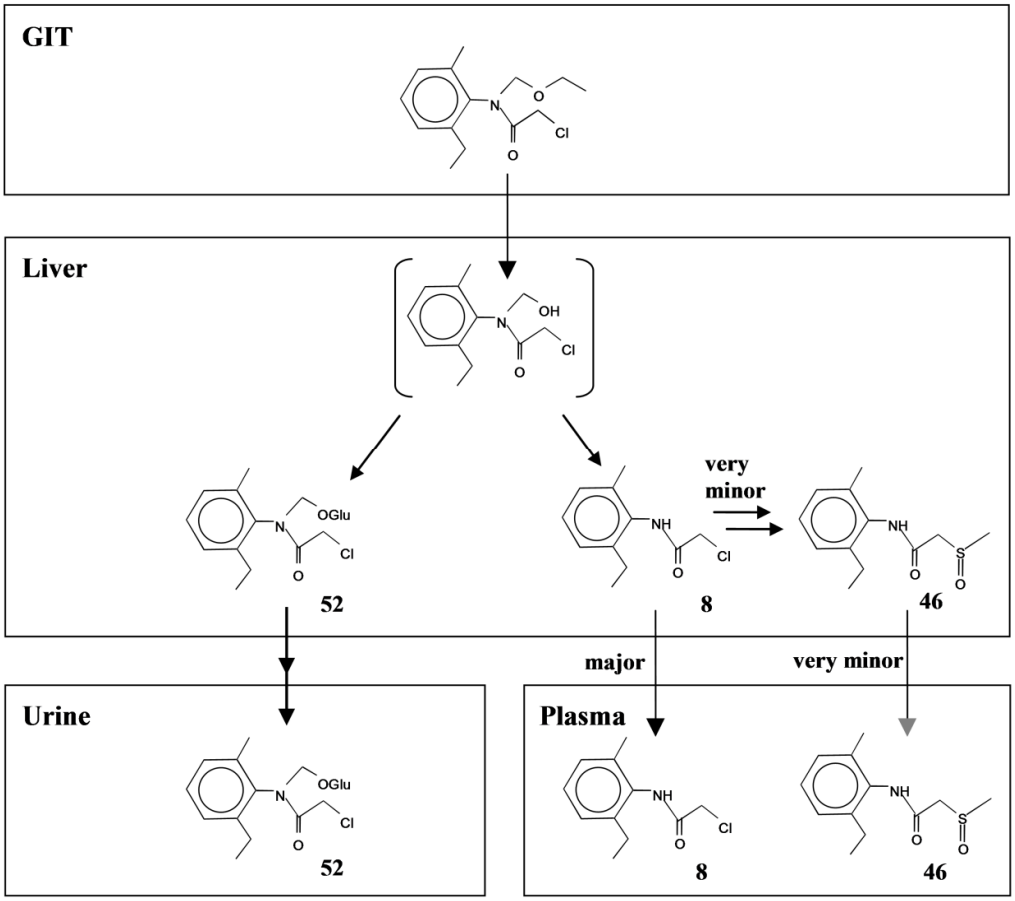
GIT: gastrointestinal tract

[a] This pathway represents a simplified version of a very complex metabolic pathway. A number of minor metabolites, including those formed from *S*-methylation, thioethers, disulfides and those formed from *S*-oxidation and/or hydroxylation of the alkyl group side-chains, are not shown in the figure. All numbers shown in figure are EU reference numbers.

* Kidney not actually analysed, but mercapturate formation can occur in the kidney based on similar compounds and then be excreted in urine.

** Formation of EU reference no. 71 from EU reference no. 36 via EU reference no. 34 was observed in in vitro experiments conducted primarily in the absence of NADPH. However, the direct precursor to EU reference no. 71, EMA (EU reference no. 34), was not identified in plasma in rat metabolism studies. Work from a different in vitro study has also shown that EMA (EU reference no. 34) can be formed directly from EU reference no. 8 (not shown). However, the vast majority of benzoquinoneimine comes from the sulfoxide (EU reference no. 46).

Fig. 3. Major metabolic pathway of acetochlor in the mouse[a]



GIT: gastrointestinal tract

[a] Glucuronides formed following hydroxylation of the alkyl group side-chains were also observed, but are not shown in this figure. All numbers shown in figure are EU reference numbers.

(b) Dermal application

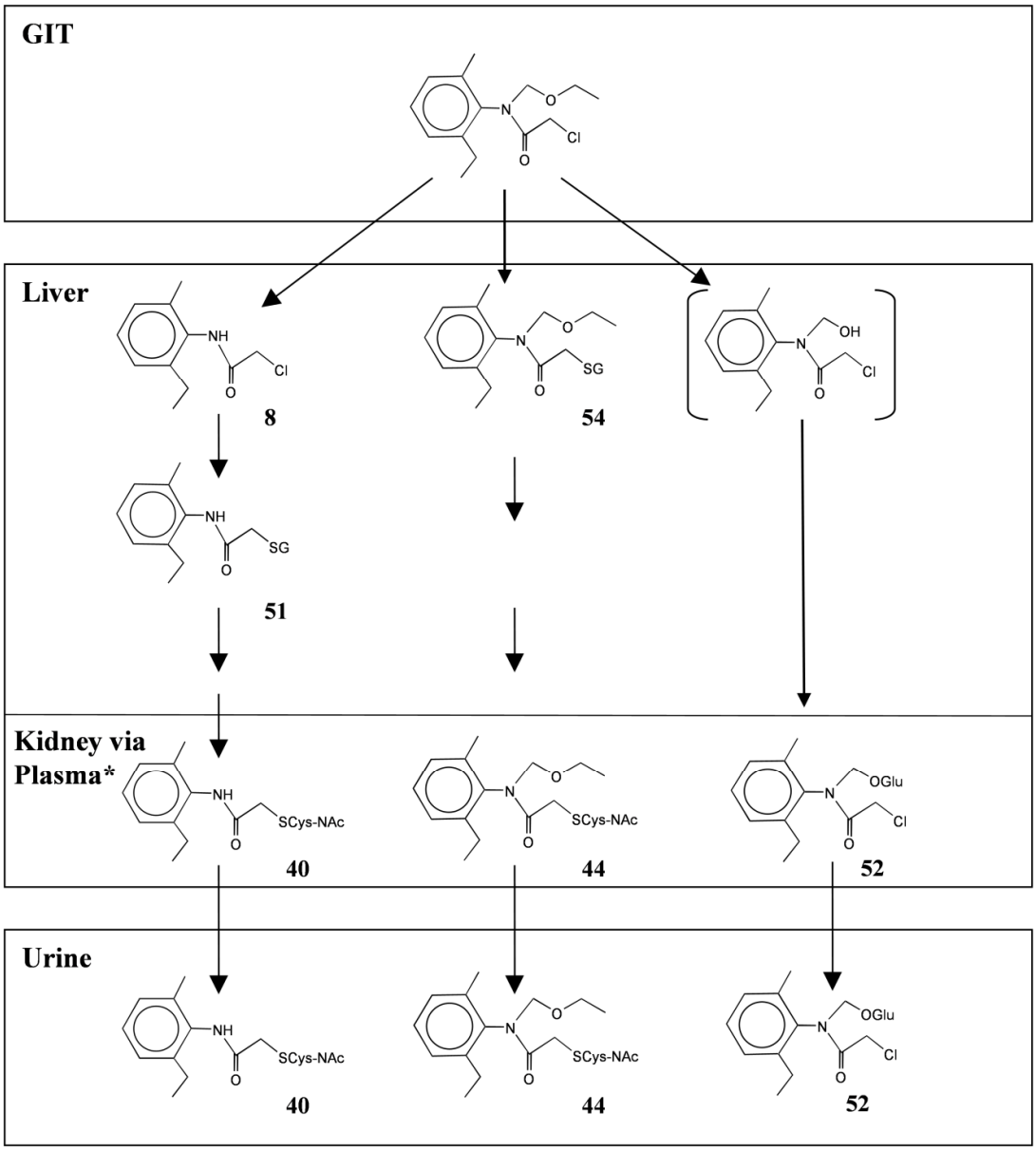
Rabbits

In an acute dermal toxicity study, undiluted acetochlor (purity 96.3%) was applied at 5000 mg/kg bw to the shaved and abraded dorsal skin of five male and five female New Zealand White rabbits, with 24-hour occluded exposure. Because 4/5 males and 4/5 females died, doses of 2500 and 3536 mg/kg bw were applied onto the intact and abraded skin of two groups of four male and four female rabbits. There was no mortality at 2500 mg/kg bw, and at 3536 mg/kg bw, 0/4 males and 1/4 females died. Clinical signs included convulsions, tremors, ataxia, bruxism, salivation, prostration, erythema, oedema and epidermal desquamation of the skin in the exposed area.

Under the study conditions utilized, the acute dermal LD_{50} for the combined sexes was calculated to be 4166 mg/kg bw. Acute dermal LD_{50} values could not be calculated for the separate sexes, but lethality data indicate that both of these values were between 3536 and 5000 mg/kg bw (Branch, 1982a).

In an acute dermal toxicity study in rats, acetochlor (purity 90.5%) was applied at 2.0 mL/kg (= 2.06 g/kg) bw to the shaved dorsal skin of five male and five female Charles River CD rats, with 24-hour occluded exposure.

Fig. 4. Major metabolic pathways of acetochlor in the rhesus monkey[a]



GIT: gastrointestinal tract

[a] A number of other minor metabolites were also observed, but are not shown in this figure. All numbers shown in figure are EU reference numbers.

* Kidney and plasma not actually analysed, but mercapturate formation can occur in the kidney based on similar compounds and then be excreted in urine.

There was no mortality. The only possible signs of systemic toxicity were pigmented orbital secretion in two rats on days 2 and/or 3 and loss of corneal lustre in one male on day 2. Dermal responses at the application site consisted of well-defined erythema (all animals on days 2–4) and exfoliation (days 4–5), with full recovery by day 7. Necropsy at day 15 showed no evidence of any treatment effect.

Under the study conditions utilized, the acute dermal LD_{50} of acetochlor in the rat was greater than 2.06 g/kg bw (Cummins, 1986a).

Table 24. Results of studies of acute toxicity of acetochlor

Species	Strain	Sex	Route	Purity (%)	LD_{50} (mg/kg bw) or LC_{50} (mg/L)	Reference
Rat	Sprague-Dawley	Male Female	Oral	96.3	2 389 (male) 1 929 (female) 2 148 (combined)	Branch (1982b)
Rat	Sprague-Dawley (CD)	Male Female	Oral	90.5	4 238 (male) 4 015 (female)	Cummins (1986b)
Rabbit	New Zealand White	Male Female	Dermal	96.3	4 166 (combined)	Branch (1982a)
Rat	Sprague-Dawley	Male Female	Dermal	90.5	> 2 060	Cummins (1986a)
Rat	Sprague-Dawley	Male Female	Inhalation	92.5	> 3.0	Bechtel (1988)
Rat	Wistar	Male Female	Inhalation	89.4	3.99 (female) > 4.46 (male)	Brammer (1989)
Rat	Wistar	Male Female	Inhalation	90.4	> 2.07	Duerden & Lewis (1990)
Rabbit	New Zealand White	Male Female	Skin irritation	96.3	Mildly irritating	Branch (1982d)
Rabbit	New Zealand White	Male	Skin irritation	89.4	Severely irritating	Barlow & Ishmael (1989)
Rabbit	New Zealand White	Male Female	Eye irritation	96.3	Minimally irritating	Branch (1982c)
Rabbit	New Zealand White	Female	Eye irritation	89.4	Minimally irritating	Pemberton & Ishmael (1989)
Guinea-pig	Hartley	Male Female	Skin sensitization	96.3	Positive (Buehler test)	Auletta (1983)
Guinea-pig	Dunkin Hartley	Male Female	Skin sensitization	89.4	Positive (maximization test)	Botham & Ishmael (1989
Mouse fibroblast	Mouse (Balb/c 3T3 clone A31)	Not applicable	Phototoxicity	96.1	Positive	Bowen (2014)

bw: body weight; LC_{50}: median lethal concentration; LD_{50}: median lethal dose

(c) Exposure by inhalation

Rats

In an acute inhalation toxicity study, five male and five female Sprague-Dawley rats were exposed (whole body) for 4 hours to an acetochlor (purity 92.5%) aerosol at a mean analytical concentration of 3.0 mg/L.

There was only one particle size measurement (mass median aerodynamic diameter [MMAD] = 2.1 µm; geometric standard deviation [GSD] = 1.8; 99.6% of the particles were < 10 µm) during the study. There was no mortality. Salivation and perinasal wetness were noted during exposure. The only observation made immediately after exposure was perioral wetness. All rats appeared normal during the post-exposure period. No gross necropsy abnormalities were noted.

Under the study conditions utilized, the 4-hour acute inhalation median lethal concentration (LC_{50}) was greater than 3.0 mg/L in rats (Bechtel, 1988).

In a second acute inhalation toxicity study, a group of five male and five female rats was exposed (nose only) for 4 hours to acetochlor (purity 89.4%) at a concentration of 4.46 mg/L. Additional groups of five females were similarly exposed to concentrations of 1.81 and 3.57 mg/L.

At 4.46 mg/L, four female rats were found dead on day 3. The remaining female and one male were killed in extremis on days 3 and 8, respectively. There were no mortalities in females exposed to 1.81 or 3.57 mg/L. Clinical signs consistent with neurological effects and respiratory tract irritation were apparent in all treated groups during and after exposure.

Under the study conditions utilized, the 4-hour inhalation LC_{50} was calculated to be 3.99 mg/L for female rats and more than 4.46 mg/L for male rats (Brammer, 1989).

In a third acute inhalation toxicity study, five male and five female Wistar rats were exposed (nose only) to acetochlor (purity 90.4%) at an analytical concentration (± standard deviation) of 2.07 (± 0.26) mg/L. The MMAD was 1.27 µm, and the GSD was 1.50. There was no mortality. All rats showed clinical signs, which included activity decrease and hunched posture, as well as some signs (lacrimation and abnormal respiratory noise) consistent with respiratory tract irritation.

Under the study conditions utilized, the 4-hour inhalation LC_{50} was greater than 2.07 mg/L in rats (Duerden & Lewis, 1990).

(d) Dermal irritation

In a primary skin irritation study with three male and three female New Zealand White rabbits, 0.5 mL aliquots of acetochlor (purity 96.3%) were applied to each of four sites (two intact, two abraded) per animal, with 24-hour occluded exposure. Skin reactions were scored at approximately 1 hour, 48 hours and 5 days after removal of the patch.

The overall mean scores for intact skin were 0.08 for erythema and 0.00 for oedema at a 24-hour exposure time.

Under the study conditions utilized, acetochlor was mildly irritating to the skin of rabbits (Branch, 1982d).

In a second primary skin irritation study, 0.5 mL samples of undiluted acetochlor (purity 89.4%) were applied to a 2.5 cm × 2.5 cm site on the left flank of each of six male New Zealand White rabbits. Exposure was for 4 hours.

At 30–60 minutes after removal of the dressing, there was severe (grade 4) erythema and slight (grade 2) to severe oedema (grade 4) at all six sites. On day 3, two sites scored 4 for erythema,

and three sites scored 4 for oedema. The primary dermal irritation index (mean of scores for 30–60 minutes and days 1, 2 and 3) was 6.13. On day 7, two sites scored 4 for erythema, whereas all six sites scored 0 for oedema. On day 21, one site scored 3 for erythema; the other five scored 0. One of six animals still showed grade 2 erythema on day 29. Signs of irritation persisting 29 days after application included thickening of the skin and increased hair growth on the application site. Histopathological examination revealed a marked increase in the number of hair follicles. One animal also showed subepithelial fibrosis.

Under the study conditions utilized, acetochlor was severely irritating to rabbit skin (Barlow & Ishmael, 1989).

The submitter stated that the test material used in the Branch (1982d) study is more representative of the technical material currently being produced.

(e) Ocular irritation

Rabbits

In a primary eye irritation study, 0.1 mL of acetochlor (96.3%) was instilled into the conjunctival sac of the right eye of each of nine New Zealand White rabbits. Three eyes (from one male and two females) were washed out after 25 seconds, and the eyes of the other six animals (three of each sex) remained unwashed after exposure.

Only conjunctival effects (which included redness, chemosis and discharge; however, the report does not break down the effects by these categories) were observed in rabbits with unwashed eyes. The mean maximum total score (at 24 hours, with a maximum possible score of 110) was 1.67, with one rabbit scoring 4 at 24 hours. All scores were 0 by day 4.

Under the study conditions utilized, acetochlor was minimally irritating to the eyes of rabbits (Branch, 1982c).

In a second primary eye irritation study, 0.01 mL of undiluted acetochlor (89.4%) was instilled in the eye of one female New Zealand White rabbit. As the rabbit showed a slight initial pain reaction and no irritation, this was followed by the instillation of 0.1 mL of undiluted acetochlor (purity 89.4%) into the eye of a second rabbit before the study proceeded to a full group of six rabbits.

There were no effects on the cornea or iris. At 1 hour, all eyes showed slight (score of 1) conjunctival redness, three eyes showed slight (score of 1) chemosis, and all eyes showed severe (score of 3) discharge. At 24 hours, one eye showed slight redness; the mean irritation score at 24 hours was 0.06 (maximum 110).

Under the study conditions utilized, acetochlor was minimally irritating to the eyes of rabbits (Pemberton & Ishmael, 1989).

(f) Dermal sensitization

In a dermal sensitization study, a group of 10 Hartley albino guinea-pigs was evaluated in a nine-induction Buehler protocol. A group of five males and five females was exposed to 0.2 mL of undiluted acetochlor (purity 96.3%) for 6 hours/day, 3 days/week, for 3 weeks. Two weeks after the last induction dose, 0.2 mL of undiluted acetochlor was applied to previously untreated areas on the guinea-pigs that received the induction doses and to three male and three female naive animals.

All 10 previously induced animals showed positive responses at 24 and 48 hours; 1/6 and 0/6 naive animals showed a positive response at 24 hours and 48 hours, respectively.

Under the study conditions utilized, acetochlor was positive as a dermal sensitizer (Auletta, 1983).

In a second dermal sensitization study, acetochlor (purity 89.4%) was evaluated in a guinea-pig maximization test. Dose levels used for induction and challenge were based on the results of a preliminary irritation study. For induction, the following three intradermal injections were made on each side of the midline of the scapular region: (1) A 1:1 mixture of Freund's Complete Adjuvant (FCA) + corn oil; (2) 10% (weight per volume [w/v]) acetochlor in corn oil; and (3) 10% (w/v) acetochlor in a 1:1 mixture of FCA + corn oil. One week later, there was topical exposure to the undiluted test substance for 48 hours under occlusive dressing. Challenge was 2 weeks later to 0.05–0.1 mL of undiluted acetochlor (shorn left flank) and to a 30% (w/v) preparation of acetochlor in corn oil (shorn right flank). Sites exposed to undiluted acetochlor showed an extreme response (18/19 positive versus 0/10 controls), whereas sites exposed to the 30% (w/v) preparation of acetochlor in corn oil showed a strong response (erythema in 13/19 versus 0/10 controls).

Under the study conditions utilized, acetochlor was positive as a dermal sensitizer (Botham & Ishmael, 1989).

(g) Phototoxicity

Acetochlor was assayed for phototoxicity to Balb/c 3T3 fibroblast cells using the neutral red uptake assay.

Based on the results of this assay, acetochlor was considered a probable phototoxicant in this in vitro test system, when tested up to the limit of toxicity, according to OECD Test Guideline 432 (Bowen, 2014).

2.2 Short-term studies of toxicity

(a) Oral administration

Mice

In a 91-day dietary toxicity study, acetochlor (purity 91.3%) was administered in the diet to 20 Swiss albino CD-1 mice of each sex per group at a dose level of 0, 800, 2000 or 6000 ppm (equivalent to 0, 120, 300 and 900 mg/kg bw per day, respectively) for 91 days. Homogeneity and stability of diets at room temperature were measured. The test substance intake was not reported. Clinical chemistry and haematological parameters were not evaluated. Urine analysis and ophthalmological examination were not performed.

No clinical abnormalities were observed throughout the course of the study. One control female mouse was found dead after 48 days on test. One high-dose female mouse was killed after 7 days on test, and one high-dose female mouse died after 12 days on test. Statistically significant decreases in feed consumption were observed in all dose groups at various intervals, but these were not considered to be compound related because the mean daily consumption of treated mice was similar to that of the untreated mice. The feed efficiency in both sexes at the various dose levels was not remarkably different from that of the control groups. In the males and females, body weights of mice in the low-dose group were comparable with those of controls. In males, a statistically significant decrease in body weight was observed in the mid-dose group compared with the control group at weeks 10 and 12, and a statistically significant decrease was observed in the high-dose group from week 1 throughout the remainder of the study. In the females, no statistically significant decrease in body weight was observed in the mid-dose group throughout the study duration. A statistically significant decrease in body weight was observed in the high-dose females from weeks 5 through 13. The changes in body weight at the high dose are considered to be test substance related.

Statistically significant changes in absolute mean organ weights included a decrease in brain weights in males at the high dose, an increase in liver weights in males at the middle and high doses and in females at the low and middle doses, an increase in kidney weights in males at the low and middle doses and a decrease in kidney weights in females at the high dose, and a decrease in gonad weights in females at the high dose. Statistically significant changes in relative mean organ weights included an increase in relative brain weights in males at the high dose, an increase in relative heart

weights in males at the middle and high doses, an increase in relative liver weights in males at the middle and high doses and in females at all dose levels (not dose related), and an increase in relative kidney weights in males at all dose levels and in females only at the middle dose level.

No test substance–related gross or microscopic pathological findings were noted in any of the mice that died or were killed during the study.

Based on the results of the 91-day dietary toxicity study in mice, the no-observed-adverse-effect level (NOAEL) was 2000 ppm (equivalent to 300 mg/kg bw per day), based on decreased body weights observed at 6000 ppm (equivalent to 900 mg/kg bw per day) (Ahmed, 1981a).

Rats

In a 4-week oral toxicity study, acetochlor (purity not reported) was administered in the diet to 10 CD (Sprague-Dawley-derived) rats of each sex per group at a dose level of 0, 300, 600, 1200, 2400, 4800 or 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) for 29 days.

Signs of reaction to treatment comprised piloerection and loss of skin elasticity, mainly observed at the two highest dose levels. One male of the highest-dose group was killed in extremis on day 12 following sustained weight loss; this death was attributed to a congenital defect of the brain unrelated to treatment. Five animals died during the blood sampling in week 4 of treatment. Two of the deaths at the highest dose may be considered to be indirectly associated with treatment. Changes in body weight, feed consumption, haematology and blood biochemistry are summarized in Tables 25 and 26.

Table 25. Consumption, body weight, haematology and blood biochemistry data for male rats

Significant findings	0 ppm	300 ppm	600 ppm	1 200 ppm	2 400 ppm	4 800 ppm	9 600 ppm
Feed consumption (% of control)	100	97	100	97	90	82	59
Water consumption (% of control)	100	97	106	106	98	89	80
Body weight gain (0–28 days, g)	198	179*	182*	183*	149**	125**	40**
Packed cell volume (%)	47	47	46	47	46	45*	45**
Haemoglobin (g%)	15.9	16.2	15.5	16.0	15.9	15.4	15.1**
MCV (μm^3)	63	62	62	63	61	62	59**
MCH (pg)	22	22	21	21	21	21	20**
Prothrombin time (s)	14	14	13.6	13.5*	13.3**	13.3**	12.9**
GGT (IU/L)	2	3	2	2	3	7**	30**
Plasma butyrylcholinesterase (IU/L)	486	555	543	490	448	523	840**
Plasma acetylcholinesterase (IU/L)	575	606	580	566	521	557	744**

GGT: gamma-glutamyltranspeptidase; IU: International Units; MCH: mean corpuscular haemoglobin; MCV: mean cell volume; ppm: parts per million; *: $P < 0.05$, **: $P < 0.01$
Source: Broadmeadow (1985)

Table 26. Consumption, body weight, haematology and blood biochemistry data for female rats

Significant findings	0 ppm	300 ppm	600 ppm	1 200 ppm	2 400 ppm	4 800 ppm	9 600 ppm
Feed consumption (% of control)	100	104	102	102	97	92	75
Water consumption (% of control)	100	108	105	108	97	111	109
Body weight gain (0–28 days, g)	87	88	85	81	67**	59**	29**
Packed cell volume (%)	48	48	47	49	48	47	45**
Haemoglobin (g%)	16.1	15.8	15.8	16.2	16.0	15.7	14.8**
MCHC (%)	34	33	33	33	33	34	33**
MCV (μm^3)	63	63	63	63	63	65	61*
MCH (pg)	21	21	21	21	21	22	20**
WBC (1 000/mm^3)	10.5	10.8	11.1	12.1	12.2	10.6	15.6**
Prothrombin time (s)	14.1	13.9	13.8	14.0	13.7	13.4**	13.0**
ALP (IU/L)	95	92	94	87	99	107	117*
GGT (IU/L)	3	3	3	3	4	11**	26**
Plasma butyrylcholinesterase (IU/L)	3 135	3 166	2 485	2 960	2 248**	2 081**	1 886**
Plasma acetylcholinesterase (IU/L)	1 858	1 872	1 589	1 592	1 368**	1 231**	1 287**
RBC acetylcholinesterase (IU/L)	2 218	1 938**	1 946**	2 185	1 755**	2 268	2 023*

ALP: alkaline phosphatase; GGT: gamma-glutamyltranspeptidase; IU: International Units; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; MCV: mean cell volume; ppm: parts per million; RBC: red blood cells; WBC: white blood cells; *: $P < 0.05$, **: $P < 0.01$
Source: Broadmeadow (1985)

Decreased body weight gain was noted in both sexes at 2400 ppm and above. Slight but statistically significant decreases in weight gain were also noted in males at 300, 600 and 1200 ppm, but were not dose related. A dose-related shortening of prothrombin time was recorded in males receiving 1200 ppm or above and in females receiving 4800 ppm or above. A marked elevation of GGT activity was observed in all animals treated with 4800 ppm or above. Plasma butyrylcholinesterase and acetylcholinesterase activities were lower than control values in females treated at 2400 ppm or above and higher than control values in males treated with 9600 ppm. Red blood cell acetylcholinesterase activity tended to be lower than control values in females, but these differences were not dose related and were considered not to be attributable to treatment. Brain cholinesterase activity was unaffected. There was a dose-related increase in relative liver and kidney weights in males and females at 2400 ppm and above. Statistically significant changes in spleen and testes weights in males and adrenal weights in females were also noted at 4800 and/or 9600 ppm. No macroscopic pathological changes were detected in rats killed after 4 weeks of treatment that could be attributed to the administration of acetochlor.

In conclusion, the results from this range-finding study indicate that the liver is a target organ of acetochlor. 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) (Broadmeadow, 1985).

In a 91-day oral toxicity study, acetochlor (purity 91.3%) was administered in the diet to 30 CD (Sprague-Dawley-derived) rats of each sex per group at a dose level of 0, 800, 2000 or 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).

Table 27. Mean body weight data in 91-day study in rats

Dietary concentration (ppm)	Body weight (g)												
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12	Week 13
Males													
0	181	225	272	310	344	365	376	398	417	433	444	460	470
800	174	215*	261*	297*	327*	353	355	370*	383*	402*	415*	431*	439*
2 000	167*	211*	251*	288*	318*	338*	348*	364*	373*	387*	403*	420*	427*
6 000	140*	169*	217*	247*	267*	286*	300*	313*	324*	343*	354*	363*	375*
Females													
0	137	156	176	191	202	213	220	228	234	241	246	253	257
800	135	151	167*	183*	193	202*	208	216	220*	226*	231*	236*	240*
2 000	135	151	168*	180*	195	201*	204*	214*	218*	224*	228*	233*	236*
6 000	123*	140*	162*	175*	185*	191*	193*	199*	204*	207*	214*	216*	218*

ppm: parts per million; * $P \leq 0.05$ (Student's *t*-test)
Source: Ahmed (1980a)

No abnormal clinical findings were observed and no animals died during the course of the study. Decreased feed consumption was noted at all dose levels. Student *t*-test analysis of the data showed that body weight was statistically significantly decreased for both sexes in all groups at several time points (Table 27). However, use of another statistical analysis, Dunnett's test, changes the interpretation of these data, especially at the low dose level in females, as the significance becomes limited only to weeks 6 and 12 compared with weeks 3, 4, 6 and 9 through 13 using Student's *t*-test.

Treatment-related changes in body weight, feed consumption, haematology, blood biochemistry and organ weights are summarized in Table 28.

A number of changes in haematological, clinical chemistry and urine analysis parameters were observed in all groups, but were not considered toxicologically significant, as the changes were small in magnitude and there was no underlying histopathology. Relative liver weight was increased by 18% in males and females at 6000 ppm, but remained without associated histopathological findings. Necropsy did not reveal any treatment-related lesions.

Based on the results of the 91-day toxicity study in rats, a NOAEL could not be identified, as effects were observed at all doses. The lowest-observed-adverse-effect level (LOAEL) was 800 ppm (equal to 53.2 mg/kg bw per day), based on marginally decreased feed consumption and body weight gain observed at the lowest dose tested (Ahmed, 1980a).

In a second 13-week oral toxicity study, acetochlor (purity 90.5%) was administered in the diet to 10 CD (Sprague-Dawley-derived) rats of each sex per group at a dose level of 0, 20, 200 or 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) for 13 weeks.

There were no treatment-related mortalities or clinical signs of toxicity. Two females (one in the control group and one in the 20 ppm group) died during routine blood sampling. Treatment-related changes in body weight gain, feed consumption, some blood biochemistry parameters and organ weights are summarized in Table 29. There was no effect on survival, and there were no treatment-related haematology, necropsy or histopathology findings. Ophthalmoscopic examination after 12 weeks of treatment did not reveal any treatment-related ocular lesions. Urine analysis parameters were not altered by treatment. The body weight gains of rats receiving 2000 ppm were significantly lower

Table 28. Body weight, haematology and blood biochemistry data and gross pathology findings in a 91-day study in rats

	Males				Females			
Significant findings	**0 ppm**	**800 ppm**	**2 000 ppm**	**6 000 ppm**	**0 ppm**	**800 ppm**	**2 000 ppm**	**6 000 ppm**
Body weight, 91 days (g)	470	439*	427*	375*	257	240*	236*	218*
Feed consumption, 91 days (g/week)	169	169	161	153*	124	126	118	110*
Haemoglobin, 91 days (g/dL)	14.4	14.6	14.5	13.8	14.6	14.0	14.6	13.5*
ALP, 91 days (IU/L)	150	141	114*	103*	67	87	53	53
ALT, 91 days (IU/L)	48	42	31*	45	46	31*	37	30*
Cholesterol, 91 days (mg/dL)	88	99	98	113*	99	106	99	104
Potassium, 91 days (mEq/L)	5.6	5.8	5.4	5.5	5.6	5.9	6.0*	6.0*
Total bilirubin, 91 days (mg/dL)	0.5	0.4	0.6	0.6*	0.6	0.5*	0.6	0.6
Albumin, 91 days (g/dL)	4.7	4.6	4.4*	4.2*	4.5	4.3	4.2	4.4
LDH, 91 days (IU/L)	392	412	410	426*	517	534	480	475
Total protein (g/dL)	7.2	7.1	6.8*	6.7*	7.2	7.1	6.9	6.7*
Absolute brain weight, 91 days (g)	2.11	2.03	2.01*	1.94*	1.86	1.89	1.83	1.88
Absolute heart weight, 91 days (g)	1.42	1.38	1.36	1.20*	0.85	0.83	0.80	0.76*
Relative brain weight, 91 days	0.005	0.005	0.005	0.006*	0.008	0.009*	0.008	0.009*
Relative liver weight, 91 days	0.033	0.030	0.033	0.039*	0.030	0.030	0.030	0.038*
Relative kidney weight, 91 days	0.007	0.007	0.008	0.009*	0.007	0.008*	0.008	0.008*
Relative gonad weight, 91 days	0.008	0.008	0.008	0.010*	0.000	0.001*	0.001*	0.001*

ALP: alkaline phosphatase; ALT: alanine transaminase; Eq: equivalents; IU: International Units; LDH: lactate dehydrogenase; *: $P < 0.05$ (Dunnett's test)
Source: Ahmed (1980a)

than those of controls during the first half of the treatment period ($P < 0.001$). Males receiving 200 ppm were similarly affected ($P < 0.05$) during this period. After 13 weeks of treatment, the body weight gains of males and females receiving 2000 ppm were, respectively, 14% and 23% lower than those of controls, and the body weight gains of males receiving 200 ppm were 7% lower than those of controls. The decreased body weights of males at 200 ppm were not considered to be adverse.

Male and female rats fed 2000 ppm acetochlor exhibited slightly increased plasma urea and cholesterol concentrations. Marginally lower plasma potassium and calcium concentrations were observed in females at 2000 ppm. The changes in clinical chemistry parameters in males and females at 2000 ppm were not considered to be adverse, as the magnitude of change was marginal and not associated with any histopathological findings. Among females receiving 2000 ppm, plasma butyrylcholinesterase and plasma acetylcholinesterase activities were low in comparison with contemporary control values. Among male rats treated at 2000 ppm, the plasma cholinesterase activities were 86% (butyryl) or 89% (acetyl) of those of control males, but the intergroup differences were not statistically significant ($P > 0.05$). The level of activity of erythrocyte acetylcholinesterase

Table 29. Body weight, blood biochemistry and gross pathology findings in a 13-week study in rats

	Males				Females			
Significant findings	**0 ppm**	**20 ppm**	**200 ppm**	**2 000 ppm**	**0 ppm**	**20 ppm**	**200 ppm**	**2 000 ppm**
Body weight gain, 0–7 weeks (g)	283	288	259*	234**	128	123	138	98**
Body weight gain, 7–13 weeks (g)	63	64	62	61	20	15	23	16
Body weight gain, 0–13 weeks (g)	345	353	321	295**	147	138	161	113**
Feed consumption, 1–7 weeks (g/week)	192	198	188	176*	145	142	147	131**
Feed consumption, 8–13 weeks (g/week)	192	192	182	174*	141	137	145	124**
Feed consumption, 1–13 weeks (g/week)	192	195	185	175**	143	139	146	128**
Urea, 13 weeks (mg/L)	36	37	35	52**	36	39	33	46*
Cholesterol, 13 weeks (mg/L)	63	68	71	83	73	69	77	88**
Potassium, 13 weeks (mEq/L)	3.6	3.5	3.7	3.8	3.6	3.5	3.6	3.3*
Calcium, 13 weeks (mEq/L)	5.0	5.0	5.0	5.1	5.2	5.2	5.2	5.1*
Plasma acetylcholinesterase, 13 weeks (IU/L)	873	810	823	773	4 288	4 608	3 898	3 277*
Plasma butyrylcholinesterase, 13 weeks (IU/L)	1 267	1 121	1 053*	1 084	4 389	4 826	4 299	3 271**
Relative liver weight, 13 weeks (%)	3.70	3.77	3.89	4.42**	3.88	3.88	3.96	4.51**
Relative kidney weight, 13 weeks (%)	0.756	0.780	0.745	0.868**	0.823	0.836	0.800	0.879

Eq: equivalents; IU: International Units; ppm: parts per million; *: $P < 0.05$; **: $P < 0.01$ (Dunnett's test)
Source: Broadmeadow (1986a)

was unaffected. The brain acetylcholinesterase activity of males receiving 2000 ppm was significantly lower than that of control males ($P < 0.001$). The brain cholinesterase activity at 2000 ppm in females was unaffected.

At the high dose level, statistically significant increases in relative liver and brain weights were observed in both sexes, a statistically significant increase in relative kidney weight was observed only in males and a statistically significant increase in relative spleen weight was observed only in females. There was no evidence of any treatment-related histopathological effects.

Based on the results of the 13-week dietary toxicity study in rats, the NOAEL was 200 ppm (equal to 16.1 mg/kg bw per day), based on statistically significant decreases in feed consumption and body weight gain (14% in males and 23% in females) observed at 2000 ppm (equal to 161 mg/kg bw per day) (Broadmeadow, 1986a).

Dogs

Acetochlor (purity 90.5%) was administered orally in gelatine capsules to groups of four male and four female Beagle dogs at a dose level of 0, 2, 10 or 60 mg/kg bw per day for 91 days. A similarly constituted control group received empty gelatine capsules only.

There was no mortality. Clinical signs of reaction to treatment were confined to dogs receiving 60 mg/kg bw per day and comprised diarrhoea, mucus in the faeces, salivation, emesis and vocalization during defecation. The incidence of these signs was higher in females than in males.

Clinical, haematology, blood biochemistry and gross pathology parameters for which treatment-related changes were observed are reported in Table 30.

Table 30. Clinical toxicology, clinical biology and gross pathology findings in a 91-day study in dogs

	Males				Females			
Significant findings	**0 mg/kg bw per day**	**2 mg/kg bw per day**	**10 mg/kg bw per day**	**60 mg/kg bw per day**	**0 mg/kg bw per day**	**2 mg/kg bw per day**	**10 mg/kg bw per day**	**60 mg/kg bw per day**
Body weight change, 1–13 weeks (kg)	2.5	2.7	2.4	1.7*	2.0	2.4	1.8	1.2*
Haematocrit, 12 weeks (%)	43	44	46	44	47	46	47	41**
Haemoglobin, 12 weeks (g/dL)	15.3	15.3	16.4	15.6	16.3	16.3	16.8	14.8**
RBC, 12 weeks ($\times 10^6$/mm^3)	6.40	6.43	6.87	6.43	7.03	6.73	6.99	6.08*
Glucose, 6 weeks (g/dL)	103	101	98	91**	99	98	100	93
Glucose, 12 weeks (g/dL)	106	106	101	92**	104	102	97	91*
ALT, 12 weeks (IU/L)	54	44	40	131*	45	36	42	92**
Relative liver weight, 13 weeks (%)	3.0	3.0	3.0	3.4*	2.9	3.1	3.1	3.4*

ALT: alanine transaminase; bw: body weight; IU: International Units; RBC: red blood cells; *: $P < 0.05$; **: $P < 0.01$ (Dunnett's test)
Source: Broadmeadow (1986b)

Low feed consumption was recorded for dogs of all groups, associated with feed withdrawal before routine clinical pathology investigations, and was more pronounced in dogs treated at 60 mg/kg bw per day. In the high-dose group, body weight gain was significantly lower than in control animals from week 2 to the end of the treatment. Ophthalmoscopic examinations did not reveal any treatment-related lesions. Urine analysis and examination of faeces for occult blood did not indicate any changes that were attributable to treatment. Haematocrit, haemoglobin concentration and erythrocyte count were reduced compared with control values in females dosed at 60 mg/kg bw per day after 12 weeks of treatment. Reduced blood glucose concentrations after 6 (males only) and 12 weeks of treatment were seen in animals dosed at 60 mg/kg bw per day when compared with controls. Plasma alanine transaminase (ALT) activity was increased slightly in the high-dose animals after 12 weeks. Other statistically significant differences from control values were not considered to be dose related or were minor in nature. The brain cholinesterase activity was not affected by treatment. Increased liver weights (relative to body weights) were observed in dogs receiving 60 mg/kg bw per day. Necropsy and histopathological examination did not reveal any treatment-related lesions.

Based on the results of the 91-day oral toxicity study in dogs, the NOAEL was 10 mg/kg bw per day, based on decreased body weight gain, clinical signs, increased relative liver weights, increased serum ALT activity and decreased blood glucose concentration in both sexes and reduced haemoglobin, haematocrit and erythrocyte counts in females observed at 60 mg/kg bw per day (Broadmeadow, 1986b).

Acetochlor (purity 91.3%) was administered orally to groups of six male and six female Beagle dogs in gelatine capsules for 119 days at a final dose level of 0, 25, 75 or 200 mg/kg bw per day. As these dogs exhibited vomiting at higher doses in an earlier pilot study, it was decided to condition the dogs at the start of the study for 3 weeks as follows: the low-dose group received 25

mg/kg bw per day for the duration of the study; the mid-dose group (75 mg/kg bw per day) received 25 mg/kg bw per day during the first week, 50 mg/kg bw per day during the second week and 75 mg/kg bw per day during the third through the 17th weeks; and the high-dose group (200 mg/kg bw per day) received 50 mg/kg bw per day during the first week, 100 mg/kg bw per day during the second week and 200 mg/kg bw per day during the third through the 17th weeks. Ophthalmoscopy was not performed.

Twelve dogs died or were killed in a moribund condition during the study, as follows: one male in the 75 mg/kg bw per day dose group and five males and six females in the 200 mg/kg bw per day group. Severe clinical signs, such as emaciation, loss of appetite, bloody diarrhoea, proteinuria, haematuria, decreased body weight and a very high mortality rate, indicated that the dose level of 200 mg/kg bw per day was too high for a meaningful toxicological evaluation. The mean feed consumption of the 25 and 75 mg/kg bw per day groups was slightly reduced (in a dose-related manner) in males compared with the controls from week 6 through to the end of the study. A slight decrease in mean feed consumption was also observed in the 75 mg/kg bw per day female group. Statistical significance was not achieved for reduced feed consumption at 25 or 75 mg/kg bw per day. The mean body weights for the dogs receiving 25 or 75 mg/kg bw per day did not show any statistically significant differences compared with the controls.

Many of the haematology and blood biochemistry parameters were statistically significantly different from control values (Table 31). The blood biochemistry parameter that was most frequently higher than the historical control range of the laboratory was ALT. ALT activities were increased by approximately 2.3-fold in males at 25 and 75 mg/kg bw per day and 2.2-fold in females at 75 mg/kg bw per day after 119 days of treatment. Relative liver weight was increased by 26% in males at 75 mg/kg bw per day and by 27% and 22% in females at 25 and 75 mg/kg bw per day, respectively. The increases in serum ALT activity and relative liver weight were not accompanied by any histopathological findings. The dose-dependent increase in relative adrenal weight in females also remained without any histopathological correlate. The histopathological findings at dose levels greater than 25 mg/kg bw per day were liver atrophy, fatty infiltration in renal tubules and hypocellularity of the bone marrow.

Based on the results of the 119-day oral toxicity study in dogs, a NOAEL could not be identified, as effects were observed at all doses. The LOAEL was 25 mg/kg bw per day, based on decreased red blood cell counts, decreased haematocrit, increased alkaline phosphatase activity, increased ALT activity and increased relative adrenal and liver weights in females (Ahmed, 1980b).

Acetochlor (purity 94.5%) was administered orally to groups of six male and six female Beagle dogs in gelatine capsules for 12 months at a dose level of 0, 4, 12 or 40 mg/kg bw per day.

All dogs survived the duration of the study, and no compound-related clinical signs were observed in any of the dogs. Decreases in feed consumption between 11% and 24% were observed in males receiving 40 mg/kg bw per day beginning in week 5 of the treatment. Absolute body weights of males and females at 40 mg/kg bw per day at month 12 were decreased by 13% and 17%, respectively, relative to the controls. Sporadic changes in clinical chemistry parameters were not considered to be of any biological significance, because most of them were borderline values, and many disappeared by the end of the study. No abnormal gross pathology findings were observed at terminal sacrifice. A statistically significant increase in absolute liver (18%) and kidney weights (18%) and a decrease in the testes to body weight ratio (60%) were observed in males receiving 40 mg/kg bw per day. Females at this dose level showed an increase in relative liver and kidney weights of 20% and 12%, respectively, but without statistical significance. A moderate diffuse and mild multifocal periportal fatty infiltration of the liver was observed in one male in each of the 12 and 40 mg/kg bw per day dose groups, respectively. Testicular atrophy was observed in all high-dose males.

The NOAEL in the 1-year toxicity study in dogs was 12 mg/kg bw per day, based on decreased body weights, decreased feed consumption, testicular atrophy, increased absolute liver and

Table 31. Clinical toxicology, haematology, blood biochemistry and gross pathology findings in a 119-day study in dogs

	Males				Females			
Significant findings	**0 mg/kg bw per day**	**25 mg/kg bw per day**	**75 mg/kg bw per day**	**200 mg/kg bw per day**	**0 mg/kg bw per day**	**25 mg/kg bw per day**	**75 mg/kg bw per day**	**200 mg/kg bw per day**
Survival, 17 weeks (%)	100	100	83	17	100	100	100	0
Body weight, 7 weeks (kg)	10.2	9.5	9.1	7.3*	8.8	8.7	7.8	6.3
Body weight, 17 weeks (kg)	10.9	10.1	9.5	–	9.4	9.4	8.0	–
Feed consumption, 7 weeks (g/day)	312	298	275	87*	256	283	223	85*
Feed consumption, 17 weeks (g/day)	307	287	295	–	252	285	230	–
RBC, 17 weeks ($\times 10^6$/mm^3)	7.00	6.83	6.32*	–	7.09	6.45*	6.50	–
Haemoglobin, 17 weeks (g/dL)	14.9	14.4	13.4*	–	15.6	13.5	14.5	–
Haematocrit, 17 weeks (pc/dL)	43.7	42.3	40.0*	–	44.7	39.8*	41.7	–
Glucose, 17 weeks (g/dL)	115	104*	102*	–	106	112	111	–
Globulin, 8 weeks (g/dL)	3.23	2.71*	2.74*	–	2.39	2.89	2.89	1.97
Globulin, 17 weeks (g/dL)	4.11	3.70*	3.64*	–	3.96	3.76	3.80	–
ALP, 8 weeks (IU/L)	95	99	101	–	88	152*	121*	112
ALP, 17 weeks (IU/L)	70	91	128	–	71	117	122*	–
ALT, 17 weeks (IU/L)	61	118*	200*	–	72	137	227*	–
Total protein, 8 weeks (g/dL)	6.73	5.85*	5.53*	–	6.50	6.22	6.03	4.45*
Total protein, 17 weeks (g/dL)	7.83	7.41	7.34*	–	7.80	7.54	7.49	–
LDH, 8 weeks (IU/L)	108	112	146*	–	161	153	135*	135*
LDH, 17 weeks (IU/L)	134	130	184*	–	162	143	137	–
Potassium, 17 weeks (mEq/L)	4.9	4.6	4.4*	–	4.6	4.5	4.7	–
BUN, 8 weeks (mg/dL)	15.4	17.1	19.1*	–	17.9	16.5	15.6	16.8
BUN, 17 weeks (mg/dL)	11.1	12.8	17.5*	–	16.9	14.4	13.8	–
Relative adrenal weight, 91 days	0.104	0.110	0.127	–	0.120	0.154*	0.168*	–
Relative liver weight, 91 days	27.18	29.61	34.38*	–	26.25	33.41*	31.95*	–
Relative spleen weight, 91 days	3.47	4.71	4.63	–	3.24	4.16	4.35*	–

ALP: alkaline phosphatase; ALT: alanine transaminase; BUN: blood urea nitrogen; bw: body weight; Eq: equivalents; IU: International Units; LDH: lactate dehydrogenase; pc: packed cells; *: $P < 0.05$; **: $P < 0.01$ (Dunnett's test)
Source: Ahmed (1980b)

kidney weights and decreased relative testes weight observed at 40 mg/kg bw per day (Ahmed, 1981b).

In a second study, acetochlor was administered orally to groups of five male and five female Beagle dogs in gelatine capsules for 52 weeks at a dose level of 0, 2, 10 or 50 mg/kg bw per day.

Two males and four females from the 50 mg/kg bw per day group were killed between weeks 39 and 51 as a result of excessive toxicity (above the maximum tolerated dose [MTD]). Clinical signs at 50 mg/kg bw per day included progressive body weight loss, dehydration, pallor, emaciation, salivation, swaying or shaking of the head and body, and stiffness and rigidity of the hindlimbs, resulting in incoordination, ataxia and/or abnormal gait, hopping and flexor reflexes and exaggerated tonic neck reflex. Salivation was occasionally seen in dogs from all groups, including controls, but was more frequently observed in dogs treated at 10 and 50 mg/kg bw per day. There were no observations of ataxia or abnormal gait in animals given 2 or 10 mg/kg bw per day. There were no treatment-related changes seen during the ophthalmoscopy, haematology, faecal occult blood or brain cholinesterase determinations. High urinary volumes, associated with low specific gravities, were recorded in dogs dosed with 50 mg/kg bw per day.

A 23–28% decrease in feed consumption was observed for females dosed with 50 mg/kg bw per day during the last 39 weeks of the study. At 10 mg/kg bw per day, a 13% decrease in feed intake was observed for females during the last 13 weeks of the study. The mean overall weight gains of females receiving 10 mg/kg bw per day (19.4% decrease compared with controls) and of males and females receiving 50 mg/kg bw per day were lower than those of their respective controls. Mean weight gains of males receiving 2 or 10 mg/kg bw per day were also low, but there was no dose–response relationship. Statistical significance for the decrease in feed intake and body weight gains was achieved only at 50 mg/kg bw per day in males and females.

Changes in biochemical parameters are shown in Table 32. High plasma ALT activities were recorded for animals treated at 10 or 50 mg/kg bw per day and were associated with high ornithine carbamyl transferase, alkaline phosphatase, GGT and/or aspartate transaminase (AST) activities. Other changes comprised high urea and creatinine concentrations after 24 and 50 weeks in animals receiving 50 mg/kg bw per day, low glucose and high cholesterol concentrations in animals from all treated groups and low triglyceride concentrations in animals treated at 10 or 50 mg/kg bw per day. At week 12, total protein concentrations were low, and at week 24, albumin concentrations were high, in females receiving 50 mg/kg bw per day.

Macroscopic examination at necropsy revealed abnormally shaped kidneys that had pale or mottled surfaces and were occasionally cystic in dogs treated at 50 mg/kg bw per day (Table 33). Histopathological examination revealed changes in brain, kidneys and testis in animals treated at 50 mg/kg bw per day. Changes in the brain were confined to the cerebellum: degeneration of the granular layer in the deeper parts of the vermis and depletion of the Purkinje cells in adjacent areas. In the kidney, there was evidence of renal toxicity, seen as hyperplasia of the collecting ducts and of the transitional epithelium of the papilla; in addition, there was cortical atrophy with fibrosis and scarring and dilatation of the Bowman's space. The cortical and collecting duct changes were considered to be potentially fatal if treatment had continued. In the testis, there was degeneration of the seminiferous tubules, maturation arrest and spermatic giant cells. Hypospermia was also noted in the epididymides. Microscopic findings at 10 mg/kg bw per day, confined to males, were interstitial nephritis and chronic vasculitis and seminiferous tubule degeneration. These changes were generally less severe than at the high dose. No treatment-related microscopic findings were observed in females receiving 10 mg/kg bw per day or in animals of either sex receiving 2 mg/kg bw per day. Fatty infiltration of the liver was not observed in this study.

The NOAEL in the 52-week toxicity study in dogs was 2 mg/kg bw per day, based on a decrease in feed consumption and body weight gain in females and changes in kidneys (interstitial nephritis) and tubular degeneration in testes in males observed at 10 mg/kg bw per day (Broadmeadow, 1989b).

Slides from the testes and epididymis of male dogs from this 52-week oral toxicity study (Broadmeadow, 1989b) were re-examined microscopically by a consulting pathologist. All of the high-dose males exhibited a treatment-related degeneration of germ cells and associated reduction of sperm in the epididymis. Germ cell degeneration was particularly prominent in the spermatid population, and especially in the elongating and elongated spermatids, which are the final maturation

phases of development. Despite the depletion and degeneration of germ cells within the seminiferous epithelium, active spermatogenesis was still ongoing in most tubules. One animal in the mid-dose group also showed partial germ cell depletion, focal tubular atrophy and increased germ cell degeneration, but this was associated with multifocal tubular necrosis with a lymphocytic infiltrate (lymphocytic orchitis). The epididymis of this animal was devoid of sperm and had multifocal lymphocytic aggregates in the interstitial tissue. The findings are consistent with lymphocytic orchitis and epididymitis, which is a condition of autoimmune etiology occasionally seen in Beagle dogs. As these findings show a different profile of changes than was seen in animals in the high-dose group, this single occurrence is not considered to be related to treatment with the test material (Creasy, 2003).

Table 32. Selected blood biochemistry parameters (selected time points) in a 52-week study in dogs

		Males				Females			
Parameter	**Week**	**0 mg/kg bw per day**	**2 mg/kg bw per day**	**10 mg/kg bw per day**	**50 mg/kg bw per day[a]**	**0 mg/kg bw per day**	**2 mg/kg bw per day**	**10 mg/kg bw per day**	**50 mg/kg bw per day[a]**
ALT (IU/L)	12	34	34	45	147**	29	36	35	115**
	24	31	29	37	86**	22	25	29	40**
	50	61	37	104	179*	34	41	183**	45
Ornithine carbamyl transferase (IU/L)	12	6.0	6.3	8.9	19.0**	5.3	5.4	4.8	12.3
	24	4.8	6.7	3.4	10.9**	7.2	6.4	5.8	6.9
	50	7.9	9.3	39.0	56.9	9.5	10.6	21.1*	9.8
GGT (IU/L)	12	3	4	4**	6**	4	4	3	4
	24	4	5	4	5*	5	4	5	6
	50	4	4	4	7*	5	6	6	7
Urea (mg%)	24	26	25	25	41**	28	30	26	43
	50	30	29	30	54**	30	27	30	58*
Creatinine (mg%)	24	0.8	0.9	0.8	1.0	0.9	0.9	0.8	1.2*
	50	1.2	1.1	1.1	1.8**	1.2	1.1	1.1	2.2*
Glucose (mg%)	12	105	92*	99	94*	105	96	93	91*
	24	121	113	113	104**	110	110	102*	95**
	50	109	112	112	105	123	113**	112**	109**
Cholesterol (mg%)	12	116	129	141	146*	133	136	145	118
	24	115	134	146	161**	132	155	140	164
	50	121	131	123	164*	149	143	174	170
Triglyceride (mg%)	12	35	36	33	51**	32	36	43*	44*
	24	22	22	27	35**	31	29	35	35
	50	39	44	49	65**	40	41	54	38
Protein (g%)	12	5.8	6.1	5.8	5.7	5.9	5.8	5.7	5.4**
Albumin (g%)	24	3.4	3.3	3.3	3.5	3.1	3.5	3.1	3.5*

ALT: alanine transaminase; bw: body weight; GGT: gamma-glutamyltranspeptidase; IU: International Units; *: $P < 0.05$; **: $P < 0.01$ (Student's *t*-test, two-sided)

[a] Three males and one female surviving at week 50.

Source: Broadmeadow (1989b)

Table 33. Significant histopathological findings in a 52-week study in dogs

		Males (n = 5)				Females (n = 5)			
Tissue	**Observation**	**0 mg/kg bw per day**	**2 mg/kg bw per day**	**10 mg/kg bw per day**	**50 mg/kg bw per day**	**0 mg/kg bw per day**	**2 mg/kg bw per day**	**10 mg/kg bw per day**	**50 mg/kg bw per day**
Brain	Degeneration of granular layer in vermis	0	0	0	4	0	0	0	3
	Depletion of Purkinje cells in vermis	0	0	0	4	0	0	0	2
Kidney	Interstitial nephritis	0	0	2	5	1	0	0	4
	Collecting duct hyperplasia	0	0	0	5	0	0	0	5
	Chronic vasculitis	0	0	3	4	0	0	0	5
	Cortical fibrosis	0	0	0	4	0	0	0	5
	Dilatation of Bowman's space	0	0	0	4	0	0	0	4
	Cortical atrophy	0	0	0	4	0	0	0	4
	Transitional cell hyperplasia	0	0	0	5	0	0	0	5
Testes	Tubular degeneration	0	0	4	5	–	–	–	–
	Maturation arrest	0	0	0	5	–	–	–	–
	Spermatid giant cell	0	1	0	4	–	–	–	–

bw: body weight
Source: Broadmeadow (1989b)

A Pathology Working Group (PWG) was convened in 2014 to review the testes from four dog studies conducted with acetochlor and to assess the potential relationship to treatment of any changes that were present. The epididymis and prostate were also examined, when present, to aid in the interpretation of the findings in the testes.

The PWG concluded that degenerative changes were observed in the seminiferous tubules of the testes of dogs given acetochlor at extremely high doses (200 mg/kg bw per day; Ahmed, 1980a) in subchronic toxicity studies conducted for up to 119 days. At 200 mg/kg bw per day, excessive toxicity (including emaciation, decreased body weights and a high rate of mortality) was observed. In these short-term studies, the NOAEL with regard to testicular histopathology was identified as 75 mg/kg bw per day.

In 1-year toxicity studies conducted in dogs, degenerative changes were present in the seminiferous tubules of the testes of all dogs at the highest doses tested, 40 mg/kg bw per day in the 12-month study (Ahmed, 1981b) and 50 mg/kg bw per day in the 52-week study (Broadmeadow, 1989b). The NOAELs with regard to testicular histopathology in the 1-year toxicity studies were 12 mg/kg bw per day in the 12-month study (Ahmed, 1981b) and 10 mg/kg bw per day in the 52-week study (Broadmeadow, 1989b).

A definitive pathogenesis of the degenerative changes observed in the seminiferous tubules could not be determined. No microscopic changes were observed in the prostate sections from the 119-day study (Ahmed, 1980b) and 12-month study (Ahmed, 1981b) that were available for examination by the PWG. Additionally, there were no treatment-related changes in the prostate weights or histopathology of the prostate for any of the studies in the final study reports. As the

prostate is very sensitive to hormonal disturbances, this provides additional support for the hypothesis that the treatment-related effect observed in the testes (tubular degeneration) was a direct testicular effect rather than a hormonally mediated effect (Hardisty, 2014).

Available toxicity studies showed testicular changes when acetochlor is administered at high doses in the dog, but not in the rat. Owing to the concern regarding the potential of chemical exposure to cause endocrine disruption, the data submitter consulted an independent scientific opinion from an expert. The expert concluded that based on the profile of changes seen in the reproductive tract of male dogs dosed with acetochlor, the testicular lesions are more likely to be due to a direct effect on the seminiferous epithelium than to any effect on the endocrine axis (Creasy, 2015).

(b) Dermal application

Rats

In a 21-day dermal toxicity study, groups of five male and five female Wistar rats were exposed to acetochlor (purity 89.4%) at 0, 0.1, 1, 10 or 100 mg/kg bw per day in olive oil applied onto the skin under occlusive dressing for 6 hours/day, 5 days/week, for 3 weeks. The control group received olive oil only. The dose range selected was based on the findings from preliminary dose range–finding studies.

There were no deaths and no clear effects on clinical condition. Upward curvature of the spine, not dose related, was observed in the test groups. Transient slight to moderate skin irritation was seen on the sites of application, but this was not dose related and was also observed in the male control group. Males treated at 100 mg/kg bw per day had slightly lower body weight gain compared with controls during the second half of the study. The magnitude of body weight change was small, and statistical significance was not achieved; therefore, it was not considered to be toxicologically significant. In females, this effect appeared in animals treated at 1 and 10 mg/kg bw per day, but not at the high dose level. There were no treatment-related effects on feed consumption. There were no treatment-related effects on haematology, clinical biochemistry, organ weights or pathology.

Based on the results of the 21-day dermal toxicity study in rats, the systemic toxicity NOAEL was 100 mg/kg bw per day, the highest dose tested (Leah, 1989).

Rabbits

Acetochlor (purity 94.5%) was applied dermally to groups of 10 male and 10 female New Zealand White rabbits, one half with intact skin and one half with abraded skin, 6 hours/day, 5 days/week, for 3 consecutive weeks at a dose level of 0, 100, 400 or 1200 mg/kg bw per day. At the end of the study, blood samples were collected from five animals of each sex per group for haematology and blood biochemistry evaluations. At termination, gross necropsy was performed, and organ weights were recorded. A select number of tissues were subjected to histopathological examination.

Eight male and seven female rabbits treated at 1200 mg/kg bw per day died between day 7 and day 19 of the study period. The clinical effects observed at the high dose level were anorexia, respiratory congestion, laboured breathing, ataxia, hypoactivity, body rigid, tonic convulsions, decreased limb tone, bradypnoea, impaired righting reflex, emaciation and hypothermia. No systemic toxicity was observed in the low- and mid-dose rabbits. Dermal irritation (including erythema, oedema, desquamation, coriaceousness, fissuring, atonia, intradermal haemorrhage, blanching, eschar and exfoliation) and associated histopathological changes of the skin were observed at all dose levels.

In the 21-day dermal toxicity study in rabbits, the systemic toxicity NOAEL was 400 mg/kg bw per day, based on mortality observed at 1200 mg/kg bw per day (Johnson, 1981).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In an oral carcinogenicity study, acetochlor (purity 94.5%) was administered to 50 Swiss-bred CD-1 albino mice of each sex per dose in the diet for 23 months at a dose level 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). Additional groups of 10 mice of each sex per dose were administered the same diets for 12 months and killed for a 1-year interim evaluation.

No unusual clinical signs were observed. Survival in males at termination was 60%, 50%, 50% and 26% at 0, 500, 1500 and 5000 ppm, respectively. In females, survival was reduced during the last months of the study at 5000 ppm and to a lesser extent at 1500 ppm (at termination, 62%, 50%, 34% and 26% at 0, 500, 1500 and 5000 ppm, respectively). Body weights for 5000 ppm males were 16% less than those of controls at week 53 and 18% less than those of controls at termination. Body weights for 1500 ppm males were 5% less than those of controls at week 53 and 2% less than those of controls at termination. Body weights for 5000 ppm females were 12% less than those of controls at week 53 and 10% less than those of controls at termination. Body weights of males and females at 500 ppm were comparable with those of controls (Table 34). A slight and transient anaemia in males and more pronounced and persistent anaemia in females at the middle and high dose levels were compound related. Other haematology changes were an increase in white blood cell counts in males at the high dose level and an increase in platelet counts in females at the middle and high dose levels. A slight and transient increase in serum liver enzymes was observed at the high dose level in males for ALT and in females for alkaline phosphatase, effects indicative of liver damage. Urine analysis parameters for treated groups were comparable with those of the control group.

Table 34. Body weights and organ weights of mice at 12 and 24 months

	Males				Females			
Significant findings	**0 ppm**	**500 ppm**	**1 500 ppm**	**5 000 ppm**	**0 ppm**	**500 ppm**	**1 500 ppm**	**5 000 ppm**
Body weight, 12 months (g)	36	32	28*	30*	27	26	27	22*
Body weight, 23 months (g)	35.50	35.88	34.72	29.29*	31.55	32.04	31.75	28.27*
Absolute liver weight, 12 months (g)	1.49	1.58	1.44	1.68	1.30	1.45	1.62*	1.53*
Absolute liver weight, 23 months (g)	1.62	2.10*	1.96*	2.52*	1.76	1.72	1.62	1.92
Absolute kidney weight, 12 months (g)	0.73	0.89*	0.78	0.81	0.46	0.54	0.55	0.41
Absolute kidney weight, 23 months (g)	0.73	1.06*	1.08*	0.87*	0.55	0.64*	0.52	0.60
Relative liver weight, 12 months	41.96	49.64*	52.17*	56.32*	48.86	55.65*	60.34*	70.99*
Relative liver weight, 23 months	45.51	58.46*	56.27*	87.09*	54.36	53.89	50.87	65.60
Relative kidney weight, 12 months	20.71	27.76*	28.00*	26.98*	17.40	20.70*	20.50*	19.17
Relative kidney weight, 23 months	21.35	29.70*	31.03*	29.66*	17.33	19.98	16.28	20.75*

ppm: parts per million; *: $P \leq 0.05$
Source: Ahmed (1983b)

At 500 ppm and above, statistically significantly increased absolute/relative kidney weights were observed in males at the interim and terminal sacrifices. Statistically significantly increased absolute/relative liver weights were observed at all dose levels in males at the interim and terminal sacrifices, with females showing significant increases only at the interim sacrifice time (Table 34).

Gross observations at necropsy included urinary tract lesions for males (all dose groups for scheduled sacrifice, high-dose group for animals that died or were killed in moribund condition) and females (mid- and high-dose groups for scheduled sacrifice and high-dose group for females that died or were killed in moribund condition), digestive tract (primarily liver) masses for males (mid- and high-dose groups for scheduled sacrifice animals), pulmonary masses for females (all dose groups for scheduled sacrifice animals and animals that died or were killed in moribund condition) and reproductive tract masses for females (high-dose group for scheduled sacrifice). A variety of other lesions and masses were observed, but were not considered treatment related. Non-neoplastic lesions likely to be treatment related are shown in Table 35. A positive trend was observed for interstitial nephritis for males and females; the incidences for the high-dose males and females were significantly increased compared with those of controls. A similar trend was also obtained for retinal degeneration in the females, although the incidence of this lesion in the high-dose group was not statistically significantly different from that of the control group.

Table 35. Non-neoplastic histopathology lesions in mice

		Males				Females			
Tissue	**Observation**	**0 ppm**	**500 ppm**	**1 500 ppm**	**5 000 ppm**	**0 ppm**	**500 ppm**	**1 500 ppm**	**5 000 ppm**
Eyes	*No. of tissues examined*	60	60	60	59	60	60	60	59
	Retinal degeneration	4	3	6	3	2††	3	1	8
Kidneys	*No. of tissues examined*	60	60	60	60	60	60	60	59
	Interstitial nephritis	30††	35	42	50**	31††	33	31	45**

ppm: parts per million; **: $P \leq 0.01$ (Fisher's exact test); ††: $P \leq 0.01$ (Cochran-Armitage trend test)
Source: Ahmed (1983b)

Some of the pathology results from this study were subsequently subjected to a comprehensive re-evaluation and/or peer review (Hardisty, 1997a,b,c). Increased incidences of histiocytic sarcomas were noted in all of the treated female groups relative to the controls, although the increase at 500 ppm was not statistically significant, and there was no clear dose–response relationship (Table 36). In female mice with histiocytic sarcoma, the uterus as well as the liver are the most commonly involved organs. Histiocytic sarcomas are rare in mice until 12 months of age and are slightly more common in females than in males. Published data concerning the incidence of histiocytic sarcomas are variable (Table 37). Because of the highly variable incidence of this tumour in untreated female control mice, the absence of precursor lesions and the lack of a clear dose–response relationship, the PWG concluded that "the marginal increase in the incidence of histiocytic sarcoma in these groups is most likely due to normal variation not associated with the dietary administration of acetochlor and [is] of no biological significance".

The incidences of lung tumours (alveolar/bronchiolar adenomas and/or carcinomas) in all of the treated female groups were greater than the incidences in the concurrent controls. The differences from controls were generally statistically significant, but no dose–response relationship was apparent (Table 36). In addition, the incidences of lung tumours in all of the treated female groups in this study were below the upper range of the incidence of spontaneous primary lung tumours reported in the literature (Tables 38 and 39), whereas the incidence in the concurrent control group was unusually low. Furthermore, there was no evidence of an increase in non-neoplastic proliferative changes

Table 36. Neoplastic histopathology lesions in mice fed acetochlor for 23 months (re-evaluation)

		Males				Females			
Tissue	**Observation**	**0 ppm**	**500 ppm**	**1 500 ppm**	**5 000 ppm**	**0 ppm**	**500 ppm**	**1 500 ppm**	**5 000 ppm**
Liver[a]	*No. of tissues examined*	50	50	50	50	50	50	50	50
	Hepatocellular adenoma	8	7	10	19*	2	0	1	5
	Hepatocellular carcinoma	4	4	4	9	0	0	0	2
	Hepatocellular adenoma/carcinoma	12	10	14	26**	2	0	1	7
Lung[b]	*No. of tissues examined*	50	50	50	50	50	50	50	50
	Alveolar/bronchiolar adenoma	7	10	11	5	1	7*	9**	7*
	Alveolar/bronchiolar carcinoma	6	3	3	3	0	4	1	6**
	Alveolar/bronchiolar adenoma/carcinoma	12	13	14	8	1	10**	10**	11**
Uterus, liver[c]	*No. of tissues examined*	–	–	–	–	50	50	50	50
	Histiocytic sarcoma	–	–	–	–	0	3	7**	6*

ppm: parts per million; *: $P \leq 0.05$; **: $P \leq 0.01$ (Fisher's exact test)
[a] Data derived from Pathology Working Group re-evaluation (Hardisty, 1997a).
[b] Data derived from Pathology Working Group re-evaluation (Hardisty, 1997b).
[c] Data derived from Pathology Working Group re-evaluation (Hardisty, 1997c).

Table 37. Historical control data for histiocytic sarcomas in female CD-1 mice

				Incidence (%)	
Source	**Time period**	**Study duration**	**No. of studies**	**Range**	**Mean**
Charles River (1995)	1981–1991	18 months	12	0–10.0	2.2
		21 months	3	0–6.0	3.8
		24 months	8	0–10.0	3.8
Charles River (2005)	1987–2000	78 weeks	25	0–15.0	2.7
		91–104 weeks	29	0–18.3	6.5
Inveresk Research (1996)	1990–1993	18 months	5	0–2.0	0.8
		24 months	8	0–10.0	4.0

(hyperplasia) or in tumour multiplicity. Therefore, the PWG concluded that the "apparent increased incidence of lung tumours in treated female mice is a spurious finding resulting from the abnormally low incidence of primary lung tumours in the control group and is of no biological significance" (Hardisty, 1997b). There was an increased incidence of hepatic carcinomas in males and females at the high dose. The incidence of hepatocellular carcinoma and hepatocellular adenoma combined was statistically significantly increased only in males given 5000 ppm acetochlor when compared with the control group. Examination of the mortality and body weight data indicated that 5000 ppm acetochlor in the diet for 23 months exceeded the MTD for male and female CD-1 mice. No significant differences in the incidence of hepatocellular neoplasms were present when acetochlor was given at 1500 ppm and below. The PWG concluded that these liver tumours were not appropriate for consideration in human risk assessment, as the increase occurred only at a dose level with high toxicity (Hardisty, 1997a).

Table 38. Historical control incidence data for lung adenomas and carcinomas in female CD-1 mice

Source	Approximate time period	Study duration	No. of studies	Incidence (%)					
				Adenoma		Carcinoma		Total[a]	
				Range	Mean	Range	Mean	Range	Mean
Maita et al. (1988)	1978–1983	24 months	11	na	14.5	na	12.1	17.5–38.8	26.6
Charles River (1995)	1981–1991	18 months	12	0–15.4	6.5	0–9.6	4.0	(3.3–20.0)	(10.5)
		21 months	6	0–10.0	6.2	0–10.0	3.1	(6.0–14.0)	(9.4)
		24 months	11	4.0–18.4	9.8	0–13.5	6.6	(12.0–23.1)	(16.4)
Charles River (2005)	1987–2000	78 weeks	25	0–16.0	7.7	0–12.0	4.3	(1.7–25.7)	(12.0)
		91–104 weeks	29	0–26.7	10.9	0–18.4	4.8	(0–35.0)	(15.7)
Life Sciences Research (1989)	1985–1988	18 months	13	0–9.6	5.4	0–9.6	4.4	(3.8–19.2)	(9.8)
Inveresk Research (1996)	1990–1993	18 months	5	0–16.0	7.6	2–10.0	4.0	(6.0–18.0)	(11.6)
		24 months	8	0–14.0	8.5	2–10.0	6.5	(12.0–20.0)	(15.0)

na: not available

[a] Only Maita et al. (1988) provided values for the total incidence of adenomas and/or carcinomas combined. The combined incidence from the other sources was estimated by adding the separate values for adenomas and carcinomas, and these estimates are enclosed in parentheses. However, this may be a slight overestimate, as a few animals are likely to have both an adenoma and a carcinoma.

Table 39. Historical control incidence data for lung adenomas and carcinomas in male CD-1 mice

Source	Approximate time period	Study duration	No. of studies	Incidence (%)					
				Adenoma		Carcinoma		Total[a]	
				Range	Mean	Range	Mean	Range	Mean
Maita et al. (1988)	1978–1983	24 months	11	na	14.6	na	18.9	17.5–43.5	33.4
Charles River (1995)	1981–1991	18 months	12	1.9–12.0	7.5	0–21.2	5.8	(3.8–23.3)	(13.3)
		21 months	7	0–26.0	11.6	0–16.7	4.9	(4.0–30.0)	(16.5)
		24 months	10	9.7–28	17.9	1.9–20.0	11.1	(15.7–38.0)	(29.0)
Charles River (2005)	1987–2000	78 weeks	25	0–26.0	11.7	0–23.2	5.2	(0–44.9)	(16.9)
		97–104 weeks	29	3.3–42.0	16.3	0–26.6	9.4	(8.3–54.0)	(25.7)
Life Sciences Research (1989)	1985–1988	18 months	13	4.0–17.3	10.6	3.3–12.0	8.6	(15.0–28.8)	(19.2)
Inveresk Research (1996)	1990–1993	18 months	5	0–16.0	12.8	1–8.0	3.6	(8.0–22.0)	(16.4)
		24 months	8	16.0–28.0	20.5	2.0–22.0	13.0	(26.0–38.0)	(33.5)

na: not available

[a] Only Maita et al. (1988) provided values for the total incidence of adenomas and/or carcinomas combined. The combined incidence from the other sources was estimated by adding the separate values for adenomas and carcinomas, and these estimates are enclosed in parentheses. However, this may be a slight overestimate, as a few animals are likely to have both an adenoma and a carcinoma.

An expert histopathological evaluation of the kidneys of CD-1 mice from a 2-year mouse study was conducted to review the classification of renal neoplasms encountered in both males and females in the study, determine whether any lesions representing precursor stages on the pathway to neoplasia were present in the treated groups of female mice and grade the spontaneous nephropathy in the female groups. The renal tubule tumours encountered in the male mouse groups were confirmed by the independent expert. The renal tumours in the male mice were clearly not dose related, as the group distribution was two, one, three and two for the 0, 500, 1500 and 5000 ppm groups, respectively. The tumour count in the female groups was confined to the 5000 ppm dose group and included two adenomas and two non-epithelial neoplasms of uncertain identity. Acetochlor exacerbated the severity of spontaneous nephropathy in the 5000 ppm females and, to a lesser extent, at the 1500 ppm dose. According to the expert, the presence of two adenomas in the 5000 ppm females does not represent a statistically significant finding. An incidence of 4% is lower than the historical control range (usually 10–15%) observed in rodent studies when a chemical is causally associated with the induction of renal tubule tumours in groups of 50 animals. The absence of any foci of atypical tubule hyperplasia, a preneoplastic lesion, in the high-dose group provides further support against the female adenomas being treatment related. Therefore, the expert considered the two adenomas in the high-dose females to represent biological variation and to be incidental to treatment (Hard, 2001).

In addition, a PWG review was performed to provide an expert histopathological evaluation of the kidney of mice used in the carcinogenicity bioassay. Specifically, the scope of work included a review of the classification of renal neoplasms encountered in the study and the determination of whether any lesions representing precursors of neoplasia were present in groups of female CD-1 mice.

The PWG concluded that the two renal adenomas observed in two 5000 ppm dose female mice in the long-term feeding study with acetochlor were not related to the treatment. They confirmed the diagnosis of two renal adenomas, but concluded that two other tumours were undifferentiated sarcomas of possibly lymphoreticular origin (Hardisty, 2001b).

In conclusion, no NOAEL could be identified, as effects were observed at all doses. The LOAEL was 500 ppm (equal to 75 mg/kg bw per day), based on increased absolute and relative kidney (both sexes) and liver weights (males only), a dose-related increase in interstitial nephritis in both sexes and retinal degeneration in females (positive trend). A treatment-related increase in the incidence of liver tumours was noted in males at 5000 ppm; however, the high dose was considered excessive, exceeding the MTD. In addition, statistically significant increases in the incidence of lung tumours in females of all dose groups and in the incidence of histiocytic sarcomas in females of the mid- and high-dose groups were observed, but were not considered to be treatment related (Ahmed, 1983b).

In a 78-week feeding study, acetochlor (purity 90.5%) was administered to 50 CD-1 mice of each sex per dose in the diet at a dose level of 0, 10, 100 or 1000 ppm (equal to 0, 1.1, 11 and 116 mg/kg bw per day for males and 0, 1.4, 13 and 135 mg/kg bw per day for females, respectively). Additional groups of 10 CD-1 mice of each sex per dose were administered the test material at the above dose levels for 52 weeks for interim sacrifice at 1 year. Urine analysis and clinical chemistry parameters were not evaluated in this study.

There were no treatment-related effects on survival, feed consumption, body weight or clinical observations. At the 50-week interval, packed cell volume, haemoglobin and erythrocyte count were slightly reduced (7–9%) in males and females receiving 1000 ppm. After 77 weeks of treatment, low erythrocyte parameters were recorded for males receiving 100 or 1000 ppm.

At 10 ppm and above, males showed statistically significant increases in absolute kidney weights; however, these findings at 10 ppm were not considered clearly adverse in the absence of other renal effects. After 78 weeks, absolute and relative kidney weights for males receiving 100 or 1000 ppm were higher than those of the controls. After 78 weeks of treatment, absolute and relative liver weights were higher for all groups of treated males compared with controls (Table 40). There

Table 40. Selected terminal organ weights in mice treated with acetochlor in the diet for 78 weeks

	Males				Females			
Organ	**0 ppm**	**10 ppm**	**100 ppm**	**1 000 ppm**	**0 ppm**	**10 ppm**	**100 ppm**	**1 000 ppm**
Terminal body weight (g)	47.0	48.3	46.1	45.3	37.4	36.4	38.1	36.7
Kidney								
Absolute (g)	0.85	0.92*	0.96**	1.12***	0.53	0.56	0.55	0.49*
Relative (%)	1.83	1.93	2.12*	2.50***	1.48	1.56	1.46	1.36
Liver								
Absolute (g)	2.4	2.9*	2.6	2.8	1.8	1.8	1.8	1.9
Relative (%)	5.07	5.96*	5.72	6.27*	4.90	5.01	4.77	5.18
Adrenals								
Absolute (mg)	3.0	3.0	3.0	3.0	7.0	7.0	7.0	7.0
Relative (%)	5.7	6.0	5.9	6.5	18.2	18.7	18.6	18.8
Brain								
Absolute (g)	0.52	0.51	0.51	0.51	0.52	0.53	0.52	0.50*
Relative (%)	1.12	1.08	1.12	1.14	1.45	1.50	1.41	1.39

ppm: parts per million; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$
Source: Amyes (1989)

were no macroscopic changes that were considered to be related to treatment, among either those animals killed or dying during the treatment period or those killed after 52 or 78 weeks of treatment. There were no non-neoplastic or neoplastic changes that were attributable to treatment in mice killed after 52 weeks of treatment. Increased kidney weights and increased incidences of tubular basophilia, interstitial fibrosis, cortical mineralization and hyaline casts were noted in males at 1000 ppm and were considered to be evidence of chronic renal nephropathy (Table 41). At the same dose level, only an increase in dilatation of cortical tubules was noted in females. A significantly increased incidence of renal tubular basophilia was also noted in males at 10 and 100 ppm. However, with the exception of two animals at 100 ppm, tubular basophilia was classified as minimal or slight in all animals, including those in the 1000 ppm group, and neither the severity nor incidence of this finding exhibited a clear dose–response relationship. Slightly increased kidney weights and an increased incidence of renal tubular basophilia in males at 10 and 100 ppm were not considered to be adverse owing to minimal severity, lack of a clear dose–response relationship, no associated histopathological findings at 10 and 100 ppm, lack of similar effects in females or in either sex at higher dose levels in a study of longer duration, and lack of corroborative renal findings.

In the lung, bronchiolar hyperplasia was observed in males at the two highest doses and in females at the low dose. It could be related to the lung tumours observed; however, there was a lack of a clear dose–response relationship. After 13, 24 or 50 weeks of treatment, the incidences of ophthalmoscopic findings for controls were similar to those for animals receiving 1000 ppm. After 76 weeks of treatment, the incidence of vacuoles in the anterior polar region of the lens was higher among females receiving 1000 ppm than among their controls ($P \leq 0.01$). As a result, the examination was extended to include all animals receiving 10 or 100 ppm. The incidences in these groups were similar to those of the controls.

There was a statistically significantly higher incidence of pulmonary adenomas in female mice at 1000 ppm compared with controls ($P < 0.05$; Table 42). No increase in the incidence of pulmonary carcinomas was seen in females. There were also higher incidences of this tumour in

Table 41. Non-neoplastic histopathological lesions in mice after 78 weeks of treatment

		Males				Females			
Tissue	**Observations**	**0 ppm**	**10 ppm**	**100 ppm**	**1 000 ppm**	**0 ppm**	**10 ppm**	**100 ppm**	**1 000 ppm**
Eye lens	*No. of tissues examined*	41	39	37	34	36	42	38	37
	Anterior polar vacuoles	10	11	12	9	7	10	12	20**
	Anterior polar opacities	2	4	1	1	5	4	0	1
Lungs	*No. of tissues examined*	50	50	50	50	50	50	50	50
	Bronchiolar hyperplasia	6	6	18**	15*	8	19*	17	11
Kidneys	*No. of tissues examined*	50	50	50	50	50	50	50	50
	Tubular basophilia	3	13*	11*	19**	7	7	5	9
	Interstitial fibrosis	8	8	14	26**	8	13	7	8
	Cortical mineralization	13	14	11	26*	1	0	0	4
	Dilatation of cortical tubules	9	11	9	15	2	8	7	12**
	Hyaline casts	9	8	13	22**	8	6	8	8

ppm: parts per million; *: $P \leq 0.05$; **: $P \leq 0.01$ (Fisher's exact test)
Source: Amyes (1989)

Table 42. Neoplastic histopathological lesions in the 78-week carcinogenicity study in mice[a]

		Males				Females			
Tissue	**Observation**	**0 ppm**	**10 ppm**	**100 ppm**	**1 000 ppm**	**0 ppm**	**10 ppm**	**100 ppm**	**1 000 ppm**
Liver	*No. of tissues examined*	50	50	50	50	50	50	50	50
	Adenoma	6	9	5	6	0	2	0	1
	Carcinoma	0	2	3	3	1	0	0	0
	Adenoma/carcinoma combined	6	11	8	9	1	2	0	1
Lung	*No. of tissues examined*	50	50	50	50	50	50	50	50
	Alveolar/bronchiolar adenoma	9	5	11	16	4	4	5	9
	Alveolar/bronchiolar carcinoma	3	3	3	4	1	0	2	2
	Adenoma/carcinoma combined	11	8	13	18	5	4	7	11
	% incidence of adenoma/carcinoma combined	22	16	26	36	10	8	14	22
Uterus, liver	*No. of tissues examined*	–	–	–	–	50	50	50	50
	Histiocytic sarcomas	–	–	–	–	2	1	0	5

ppm: parts per million
[a] Data derived from Pathology Working Group re-evaluation (Hardisty, 1997b).

females at 10 and 100 ppm and in males at 100 and 1000 ppm, but these incidences were not statistically significant. A positive trend with dose was observed for pulmonary adenoma in males ($P < 0.01$) and females ($P < 0.05$). These lung tumours were re-evaluated by the PWG, which concluded that the apparent increase in incidence with treatment is of no biological significance because of the lack of a dose–response relationship, the absence of precursor proliferative changes, the lack of an increase in tumour multiplicity with increasing dose and the comparable incidence of

lung tumours in the treated groups with published incidences of primary lung tumours in untreated control male and female CD-1 mice (Hardisty, 1997b).

In conclusion, the NOAEL in the 78-week dietary study in mice was 10 ppm (equal to 1.1 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 mg/kg bw per day). The NOAEL for carcinogenicity was 1000 ppm (equal to 116 mg/kg bw per day), the highest dose tested (Amyes, 1989).

A PWG review was performed on the lungs from all animals from two long-term mouse feeding studies with acetochlor to determine the incidence of pulmonary neoplasms using currently accepted nomenclature and diagnostic criteria and to discuss the relevance, if any, of these tumours for human risk assessment. The results of the PWG review of primary lung tumours from male and female mice in these two studies indicate that the apparent increase in incidence with treatment is of no biological significance. This conclusion is based on the lack of dose–response relationship, the absence of precursor proliferative changes, the lack of an increase in tumour multiplicity with increasing dose and the comparable incidence of lung tumours in treated groups with published incidences of primary lung tumours in untreated control male and female CD-1 mice (Hardisty, 1997b).

A PWG review was performed to confirm the incidence of histiocytic sarcoma of the uterus in female mice from two long-term feeding studies with acetochlor and to discuss the relevance, if any, of these tumours for human risk assessment. The overall weight of evidence indicated that the higher incidence of histiocytic sarcoma observed in female mice given 1000, 1500 and 5000 ppm acetochlor was not clearly related to dietary administration of acetochlor. The lack of increasing incidence with increasing dose, the absence of precursor lesions and the variable incidence known to occur in untreated control female mice are consistent with the interpretation that the marginal increase in the incidence of histiocytic sarcoma in these groups is most likely due to normal variation not associated with the dietary administration of acetochlor and is of no biological significance (Hardisty, 1997c).

The incidences of lung tumours and histiocytic sarcomas observed in the two dietary mouse carcinogenicity studies conducted with acetochlor were re-evaluated by a Scientific Advisory Group organized by the data submitter. The conclusion of the Scientific Advisory Group is that neither the lung tumour incidence nor the incidence of histiocytic sarcomas noted in the two mouse studies is considered an indicator for the carcinogenic potential of acetochlor (Gopinath, 2009).

Rats

In an oral carcinogenicity study, acetochlor (purity 94.5%) was administered to 70 Sprague-Dawley rats of each sex per dose in the diet for 27 months for males and 24 months for females at a dose level of 0, 500, 1500 or 5000 ppm (equal to 0, 22, 69 and 250 mg/kg bw per day for males and 0, 30, 93 and 343 mg/kg bw per day for females, respectively). Ten rats of each sex per dose were killed after 12 months for a 1-year interim evaluation.

Some of the effects at the high dose are not discussed here, as the high dose exceeded the MTD. No ophthalmological changes or clinical signs of toxicity were observed that were considered to be related to treatment. The female rats were killed before the end of the 24th month on test (week 103) because the survivors in at least one female group had decreased to less than 25% of the original number of animals per group. There were enough males in each group, however, to allow the study to proceed to 27 months. The following statistically significant differences were observed in the mean weekly body weight data for the male rats (Table 43): the low-dose group showed a decrease at weeks 9, 12 and 115; and the mid-dose group showed a decrease at weeks 2 through 115. The following statistically significant differences were observed in the mean weekly body weight data for the female rats: the low-dose group showed an increase at week 101; and the mid-dose group showed a decrease at weeks 31, 39, 41, 47 through 53, 75 through 85 and 97. There was a statistically significant decrease in feed consumption by males and females that was considered to be treatment related

Table 43. Survival, body weight and haematology data in a 2-year study in rats

	Males				Females			
Significant findings	**0 ppm**	**500 ppm**	**1 500 ppm**	**5 000 ppm**	**0 ppm**	**500 ppm**	**1 500 ppm**	**5 000 ppm**
Survival, termination (%)	32	33	45	25	42	32	43	18
Body weight, start (g)	174.5	170.6	170.4	172.1	147.0	144.6	147.9	146.5
Body weight, termination (g)	745.4	640.9*	618.7*	479.8*	449.5	503.4	431.5	308.1*
Haemoglobin, 18 months (g/dL)	14.2	14.2	15.1	14.8	13.9	14.1	13.3	11.4*
Haematocrit, 18 months (pc%)	41.8	41.3	44.7	43.0	41.2	41.0	40.5	34.8*
WBC, 24 months ($\times 10^3/mm^3$)	36.0	25.4	25.2	24.9	34.0	28.3	26.7	16.9*

pc: packed cells; ppm: parts per million; WBC: white blood cells; *: $P \leq 0.05$ (Dunnett's test)
Source: Ahmed (1983a)

because it occurred at frequent intervals throughout the study. Statistically significant changes in the blood chemistry parameters were not considered to be related to treatment because they were not consistent over time and/or there was no apparent dose–response relationship. No treatment-related effects were observed in urine analysis parameters evaluated during the study.

The mid- and high-dose groups (male and female) showed a dose-related increase in the kidney to body weight ratio at months 12 and 24. Most of the other changes observed were a reflection of the corresponding changes in body weight and were not considered to be treatment related because of the lack of corroborative histopathology.

Several lesions appeared to be increased in the test groups when compared with the control animals. Microscopic lesions were found only during the second year of treatment. With respect to non-tumour histopathology, there was a dose-related increase in polyarteritis in arteries and testes, particularly in dead or moribund male rats, from 12 months to the termination of the study (Table 44). Polyarteritis is a disease process that occurs in rats and is believed to result from an immunopathological mechanism related to the deposition of immune complexes within affected vessels. In females, liver necrosis showed a statistically significant positive trend and statistically significantly increased incidence in the high-dose group. In males, liver necrosis was not observed. This increase in liver necrosis in females was more pronounced in animals either dead or moribund from 12 months to the termination of the study as well as in the high-dose females killed at the end of the study. Cardiac thrombosis and peripheral neuropathy for females showed statistically significant positive trends. This resulted in increased incidences of these findings at the high dose compared with the control group, but the increases were not statistically significant. In lungs, alveolar histiocytosis and interstitial pneumonia in females were dose-relatedly increased. The weights of lungs were not determined in this study. Other findings were fibrosis and gastritis in the stomach and inflammation of the tongue in females at the high dose.

The treatment-related neoplastic histopathological finding are reported in Table 45. To ensure accuracy and consistency of the diagnoses, some of the pathology results were subsequently subjected to a re-evaluation and/or peer review (Hardisty, 1997a; Ribelin, 1987).

Treatment-related neoplastic findings were noted in the liver, nasal turbinate mucosa and thyroid. There were increased numbers of hepatocellular adenomas and carcinomas in males and females at 5000 ppm in the presence of markedly decreased body weights and increased mortality. During the first year, there were no increases in hepatic tumours.

Table 44. Non-neoplastic histopathological lesions in a 2-year study in rats

		Males				Females			
Tissue	**Observation**	**0 ppm**	**500 ppm**	**1 500 ppm**	**5 000 ppm**	**0 ppm**	**500 ppm**	**1 500 ppm**	**5 000 ppm**
No. of animals examined		70	70	70	70	70	70	70	70
Liver	Necrosis	7	2	4	5	1	8	3	12**
Lung	Alveolar histiocytosis	5	5	6	6	0	1	1	9**
	Interstitial pneumonia	6	5	5	7	6	4	9	10
Tongue	Inflammation	3	1	3	1	1	0	3	8**
Kidneys	Cyst	0	0	1	1	0	2	2	5
Testes	Polyarteritis	7	11	12	17	–	–	–	–
Heart	Thrombosis	2	3	2	1	0	0	0	4
Arteries	Polyarteritis	3	3	3	10	0	0	0	0
Peripheral nerve	Neuropathy	1	0	1	1	0	0	0	4
Stomach	Fibrosis	13	10	13	14	4	5	7	12
	Gastritis	8	3	2	2	0	1	1	3

ppm: parts per million; **: $P \leq 0.01$ (Chi-squared test)
Source: Ahmed (1983a)

Table 45. Neoplastic histopathological lesions in a 2-year study in rats

		Males				Females			
Tissue	**Observation**	**0 ppm**	**500 ppm**	**1 500 ppm**	**5 000 ppm**	**0 ppm**	**500 ppm**	**1 500 ppm**	**5 000 ppm**
Liver[a]	*No. of tissues examined*	60	60	60	60	60	60	60	60
	Hepatocellular adenoma	2	1	1	6	0	1	1	3
	Hepatocellular carcinoma	1	3	3	6	0	0	0	2
	Hepatocellular adenoma/carcinoma	3	4	4	11**	0	1	1	5*
Nasal turbinates[b]	*No. of tissues examined*[c]	69	70	69	69	69	68	70	69
	Papillary adenoma	0[††]	1	6*	18**	0	0	2	1
	Papillary adenocarcinoma	0[†]	0	0	2	0	0	0	0
Thyroid	*No. of tissues examined*	59	60	60	60	60	60	60	60
	Follicular adenoma	0[†]	0	3	5*	2	0	0	3
	Follicular carcinoma	0	1	0	2	0	0	0	0

ppm: parts per million; *: $P \leq 0.05$; **: $P \leq 0.01$ (Fisher's exact test); [†]: $P \leq 0.05$; [††]: $P \leq 0.01$ (Peto trend test)
[a] Data derived from Pathology Working Group re-evaluation (Hardisty, 1997a).
[b] Data derived from histopathology re-evaluation (Ribelin, 1987).
[c] Includes interim kill animals, as nasal tumours were noted in some animals at 12 months.
Source: Ahmed (1983a)

Interstitial cell tumours of the testes exhibited a dose-related increase in the male test groups compared with the male control group. The overall composite percentage of interstitial cell tumours was as follows: control, 2.8%; low dose, 5.7%; middle dose, 5.7%; and high dose, 10%. Most of these tumours appeared in the terminal sacrifice group of animals. The historical control data show a 2/291 (0.7%) incidence of interstitial tumours. The Peto trend test for interstitial tumours was not statistically significant.

In the thyroid gland, follicular cell adenomas exhibited a dose-related increase in the male mid-dose and high-dose test groups compared with the male control group. The overall incidence of follicular cell adenomas in the males was as follows: control, 0%; low dose, 0%; middle dose, 4.3%; and high dose, 7.1%. These tumours were evenly distributed between the terminal sacrifice animals and the dead and moribund animals from 12 months to termination. There were no follicular adenomas in the historical control data, and two follicular carcinomas were observed in a high-dose male; therefore, thyroid tumours in males were considered to be a compound-related effect.

An increased incidence of nasal tumours (primarily papillary adenomas) was noted at 1500 and 5000 ppm, predominantly in males, and is considered to be treatment related. A slight and not statistically significantly increased incidence of mucosal inflammation was also noted in the nasal turbinates of high-dose males. The inflammation was of mild severity and occurred in a different region of the turbinates than that of the tumours and thus should not be considered related to the development of papillary adenomas.

Several other tumours from various organs appear to have a dose-related increase; however, these were not considered to be toxicologically significant either because of the small number present or because they did not exhibit consistent changes over time or between sexes or a dose–response relationship.

In conclusion, the LOAEL for systemic toxicity in this carcinogenicity study in rats was 500 ppm (equal to 22 mg/kg bw per day), based on decreased body weight in males (14% compared with controls). A NOAEL was not identified in this study. The NOAEL for carcinogenicity was 500 ppm (equal to 22 mg/kg bw per day), based on an increased incidence of nasal tumours at 1500 ppm (equal to 69 mg/kg bw per day) and above (Ahmed, 1983a).

In a second oral carcinogenicity study, acetochlor (purity 96.1%) was administered to 70 Sprague-Dawley rats of each sex per dose in the diet for 24 months at a dose level 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 mg/kg bw per day for females, respectively). Ten rats of each sex per dose were killed after 12 months for a 1-year interim evaluation.

Survival was not affected by treatment. Reduced body weights were observed in animals receiving 1000 ppm. A statistically significant decrease in body weight of males (5–15%) was observed at 1000 ppm, whereas the difference was not statistically significant for females in the same dose group. The weight differences in females in the 1000 ppm dose group were less than 7%. No treatment-related effects on clinical signs, urinary parameters, haematology or ophthalmoscopic examination were observed in the study. Statistically significant differences in clinical chemistry parameters (Table 46) that were considered to be relevant and related to treatment were increased GGT activity (in high-dose males at months 18 and 24), cholesterol level (high-dose males at month 24) and total bilirubin level (in high-dose females at month 24). There were no gross findings in animals dying spontaneously or killed at sacrifice that were considered to be related to treatment.

Selected treatment-related non-neoplastic histopathological findings are presented in Table 47. Fatty infiltration between the muscles of the tongue was significantly increased in high-dose females. It is a common spontaneous occurrence in aged, obese animals. The fat occurs in a patchy distribution, and the amount encountered depends upon the point of sectioning. There was no dose–response relationship, and the finding was considered to be without meaningful significance.

Table 46. Survival, body weight, blood biochemistry and organ weight data in rats

	Males				Females			
Significant findings	**0 ppm**	**40 ppm**	**200 ppm**	**1 000 ppm**	**0 ppm**	**40 ppm**	**200 ppm**	**1 000 ppm**
Survival, termination (%)	47	40	37	38	40	43	43	48
Body weight, 23 months (g)	820.2	780.9	787.5	700.6**	505.8	522.1	527.5	469.6
GGT, 18 months (IU/L)	0.94	1.6	0.49	3.8*	4.1	3.7	3.9	4.0
GGT, 24 months (IU/L)	1.4	1.6	5.3	8.4**	0.92	1.2	1.8	1.4
Cholesterol, 24 months (mg/dL)	91.8	109	80.3	153*	50.2	56.3	58.6	73.4
Total bilirubin, 24 months (mg/dL)	0.08	0.09	0.09	0.13	0.11	0.13	0.10	0.51**
Relative liver weight, 24 months (%)	2.941	3.117	2.908	3.479[†]	3.260	2.903	2.975	3.096

GGT: gamma-glutamyltranspeptidase; IU: International Units; ppm: parts per million; *: $P \leq 0.05$; **: $P \leq 0.01$ (Dunnett's test); [†]: $P \leq 0.05$ (Mann-Whitney statistic and Bonferroni inequality)
Source: Naylor & Ribelin (1986)

Table 47. Non-neoplastic histopathological lesions in a 24-month study in rats

		Males				Females			
Tissue	**Observation**	**0 ppm**	**40 ppm**	**200 ppm**	**1 000 ppm**	**0 ppm**	**40 ppm**	**200 ppm**	**1 000 ppm**
Liver	*No. of tissues examined*	70	69	70	70	70	70	70	70
	Foci of cellular alteration	17[†]	14	13	25	19	18	14	22
Kidneys	*No. of tissues examined*	70	69	70	70	70	70	70	70
	Tubular casts/cysts/dilatation	50	59	59	64**	38	41	37	44
Tongue	*No. of tissues examined*	67	67	68	68	69	68	63	69
	Fatty infiltration	3	10	11	5	11	17	6	28**

ppm: parts per million; **: $P \leq 0.01$ (Fisher's exact test with Bonferroni inequality); [†]: $P \leq 0.05$ (Peto trend test)
Source: Naylor & Ribelin (1986)

Also increased were hepatic foci of altered cellular architecture in high-dose males and females (Peto trend test) and hepatic neoplastic nodules and thyroid follicular adenomas in high-dose females. None of these changes was statistically significantly increased over control incidence.

Some of the neoplastic findings were subjected to comprehensive re-evaluation and/or peer review (Hardisty, 1997b). The discussion here reflects the results of the most recent evaluation and may differ from the original study report. Thyroid C-cell hyperplasia was significantly increased in high-dose males that were killed at the end of the study. This lesion was also considered unrelated to treatment, as it was increased only in terminally killed animals, it was not increased in females, the degree was not severe, and there was no clear dose–response relationship.

The most important microscopic finding that was considered to be related to treatment was the occurrence of papillary adenomas of the nasal mucosa (Table 48). The liver samples from this study were re-evaluated by the PWG (Hardisty, 1997a), as an increased incidence of liver tumours was noted at 5000 ppm (Ahmed, 1983a). There was a slight increase in the incidence of hepatocellular adenomas in high-dose females, but this was not statistically significant. These liver tumours were not considered to be treatment related, because they lacked statistical significance and no evidence of

Table 48. Neoplastic histopathological lesions in a 24-month study in rats

Tissue	Observations	Males				Females			
		0 ppm	**40 ppm**	**200 ppm**	**1 000 ppm**	**0 ppm**	**40 ppm**	**200 ppm**	**1 000 ppm**
Liver[a]	*No. of tissues examined*	60	60	60	60	60	60	60	60
	Hepatocellular adenoma	0	3	1	2	0	1	1	5
	Hepatocellular carcinoma	1	1	1	1	1	1	0	1
	Hepatocellular adenoma/ carcinoma	1	4	2	3	1	2	1	6
Nasal turbinates	*No. of tissues examined*[b]	70	69	70	70	70	70	70	70
	Papillary adenoma	1	0	0	12**	0	0	0	19**

ppm: parts per million; **: $P \leq 0.01$ (Fisher's exact test)
[a] Data derived from Pathology Working Group re-evaluation (Hardisty, 1997a).
[b] Includes interim kill animals, as nasal tumours were noted in some animals at 12 months.
Source: Naylor & Ribelin (1986)

increased liver tumours was observed at 1500 ppm (Ahmed, 1983a) or at 1750 ppm (Broadmeadow, 1989a).

In conclusion, the NOAEL for systemic toxicity in this 24-month dietary study in rats was 200 ppm (equal to 9.4 mg/kg bw per day), based on decreases in body weight in males, elevated total bilirubin level in females and elevations of GGT activity and cholesterol level in males observed at 1000 ppm (equal to 47.5 mg/kg bw per day). The NOAEL for carcinogenicity was 200 ppm (equal to 9.4 mg/kg bw per day), based on an increased incidence of papillary adenomas of the nasal mucosa at 1000 ppm (equal to 47.5 mg/kg bw per day) (Naylor & Ribelin, 1986).

In a third oral carcinogenicity study, acetochlor (purity 90.5%) was administered to 50 Sprague-Dawley rats of each sex per dose in the diet for 24 months at a dose level 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). An interim sacrifice was conducted on 10 animals of each sex per group at the low and intermediate doses and on 20 animals of each sex per group for the control and high-dose groups after 12 months.

There were no treatment-related effects on survival, clinical signs of toxicity, clinical chemistry parameters, urine analysis, macroscopic findings or palpable swellings. The overall feed intake of males and females receiving 1750 ppm was, respectively, 13% and 8% lower than that of controls. These differences were statistically significant overall ($P < 0.001$). The overall body weights of males and females receiving 1750 ppm were, respectively, 12% and 33% lower than those of controls. These intergroup differences were statistically significant ($P < 0.05$). The feed conversion ratios (amount of feed consumed per unit of body weight gain) of rats receiving 1750 ppm were slightly higher than those of controls, indicating a reduced efficiency of feed utilization. In the latter phase of the study, the ophthalmoscopic examination after 76 and 101 weeks of treatment indicated that a high proportion of females receiving 1750 ppm had hyperreflection of the ocular fundus (Table 49). In the majority of affected animals, this lesion was bilateral. Examination after 76 weeks, and subsequently, indicated a slightly higher incidence of foci or plaques in the vitreous body or on the posterior capsule of the lens in males receiving 1750 ppm when compared with control males. In the majority of affected animals, this lesion was bilateral.

Low mean platelet counts after 24 weeks of treatment in males receiving 175 or 1750 ppm were considered to be a consequence of a raised mean count in controls caused by two particularly high values. These intergroup differences were not attributed to treatment. After 102 weeks of treatment, the mean cell volume in males and females fed 1750 ppm acetochlor was reduced

Table 49. Selected ophthalmological findings (week 76) in a 24-month study in rats

Finding	Males				Females			
	0 ppm	18 ppm	175 ppm	1 750 ppm	0 ppm	18 ppm	175 ppm	1 750 ppm
Hyperreflection of ocular fundus	0	2	4	1	5	4	6	24**
Vitreous body, foci/opaque spots	3	1	1	11*	3	2	3	0
Lenticular plaques/foci	4	1	4	12*	3	2	4	0

ppm: parts per million; *: $P \leq 0.05$; **: $P \leq 0.01$ (Student's *t*-test, two-sided)
Source: Broadmeadow (1989a)

(decreases of 7% and 10%, respectively) compared with controls. Other parameters that showed a statistically significant difference from controls were minor and were considered not to be adverse.

After 52 weeks of treatment, there were increased incidences of adenomas of the nasal epithelium in both male ($P < 0.05$) and female ($P < 0.01$) rats that had received 1750 ppm; these tumours were accompanied by statistically significantly increased incidences of hyperplasia of the nasal epithelium in animals treated with 1750 ppm. There were no other changes associated with treatment at 52 weeks (Tables 50 and 51).

Table 50. Non-neoplastic lesions in a 24-month study in rats

Tissue	Observation	Males				Females			
		0 ppm	18 ppm	175 ppm	1 750 ppm	0 ppm	18 ppm	175 ppm	1 750 ppm
Week 52									
Nose	*No. of tissues examined*	20	10	10	20	18	9	10	16
	Purulent rhinitis	0	0	0	2	0	0	0	0
	Epithelial hyperplasia	0	0	0	11**	0	0	0	13**
Week 104									
Nose	*No. of tissues examined*	50	49	50	50	50	50	49	49
	Purulent rhinitis	0	1	3	4	1	1	2	1
	Epithelial hyperplasia	0	0	0	25**	0	0	0	28**
	Squamous metaplasia of olfactory epithelium	0	0	0	4	0	0	1	0
Eye	*No. of tissues examined*	48	43	46	49	49	48	48	45
	Degeneration of outer retinal layer	2	1	2	7	13	7	14	24*
Kidney	*No. of tissues examined*	50	50	50	50	50	50	50	49
	Pelvic epithelial hyperplasia	6	7	10	22**	4	7	9	14**

ppm: parts per million; **: $P \leq 0.01$ (Fisher's exact test)
Source: Broadmeadow (1989a)

There was an increased incidence of hyperplasia, progressing to adenoma, of the nasal epithelium observed in the majority of animals at 1750 ppm. After 104 weeks of treatment, two males and one female had carcinoma of the nasal epithelium. Nasal olfactory hyperplasia and polypoid adenoma were also observed in the interim kill animals at 52 weeks. No nasal hyperplasia or adenoma was observed in the other treatment groups or in the controls. At 1750 ppm, treatment-related

Table 51. Neoplastic lesions in a 24-month study in rats

		Males				Females			
Tissue	**Observation**	**0 ppm**	**18 ppm**	**175 ppm**	**1 750 ppm**	**0 ppm**	**18 ppm**	**175 ppm**	**1 750 ppm**
Week 52									
Nose	*No. of tissues examined*	20	10	10	20	18	9	10	16
	Adenoma of nasal epithelium	0	0	0	5*	0	0	0	8**
Week 104									
Nose	*No. of tissues examined*	50	49	50	50	50	50	49	49
	Adenoma of nasal epithelium	0	0	0	30**	0	0	0	28**
	Carcinoma of nasal epithelium	0	0	0	2	0	0	0	1
Thyroid	*No. of tissues examined*	50	50	48	50	50	50	50	49
	Follicular cell adenoma	2	1	2	5	1	1	3	5

ppm: parts per million; *: $P \leq 0.05$; **: $P \leq 0.01$ (Fisher's exact test)
Source: Broadmeadow (1989a)

increases in the incidence of squamous metaplasia of the olfactory epithelium and purulent rhinitis were also observed in male rats. There was a slightly increased incidence of thyroid follicular cell adenoma in females at 1750 ppm. In females fed 1750 ppm acetochlor, there was an increased incidence of degeneration of the retinal outer layer, which was consistent with the ophthalmological findings. The foci or plaques in the vitreous body or on the posterior capsule of the lens in males at this dose level were not associated with any other histopathological findings.

Two rare tumours were also originally reported in animals of the high-dose group. A benign chondroma of the femur was reported in one male that died during the study and in one female that survived to week 104, and a basal cell tumour was reported in the forestomach of one male and one female that died during the study. However, a subsequent PWG re-evaluation of these lesions indicated that the original diagnoses were erroneous (Hardisty, 2001a). The femur lesions were actually cartilaginous hyperplasia, which was also observed in one control animal. The forestomach lesions were not basal cell tumours, but the more common squamous cell carcinomas, and were not considered to be treatment related.

In conclusion, the NOAEL for systemic toxicity was 175 ppm (equal to 6.4 mg/kg bw per day), based on reduced body weight and feed consumption, changes in the eyes, reduced blood cell parameters and increased incidence of focal hyperplasia in the nasal epithelium observed at 1750 ppm (equal to 66.9 mg/kg bw per day). The NOAEL for carcinogenicity was 175 ppm (equal to 6.4 mg/kg bw per day), based on adenomas and carcinomas of nasal epithelium and thyroid follicular cell adenoma at 1750 ppm (equal to 66.9 mg/kg bw per day) (Broadmeadow, 1989a).

Nasal tumours were observed in the three carcinogenicity studies in rats and also in the two-generation reproductive toxicity study in rats (see section 2.5 below; Milburn, 2001). The incidences of the tumours are summarized in Table 52.

A PWG review was performed on the livers from all animals from the five long-term rat and mouse feeding studies with acetochlor in order to determine the incidence of hepatic neoplasms using currently accepted nomenclature and diagnostic criteria and to discuss the relevance, if any, of these tumours for human risk assessment.

Table 52. Comparison of acetochlor dose with nasal tumour incidence in reproductive toxicity and carcinogenicity studies with acetochlor

			Males		Females		
Study type	**Dietary concentration (ppm)**	**Generation**	**Dose (mg/kg bw per day)**	**Tumour incidence (%)**	**Dose (mg/kg bw per day)**	**Tumour incidence (%)**	**Reference**
Reproduction	200	F_0	18.6	0	21.5	0	Milburn (2001)
		F_1	18.6	0	21.5	0	
	600	F_0	57.0	0	64.6	0	
		F_1	57.0	12	64.6	4	
	1 750	F_0	166	15	199	23	
		F_1	166	31	199	65	
Oncogenicity	500	–	22	1	30	0	Ahmed (1983a)
	1 000	–	47.5	17	60	27	Naylor & Ribelin (1986)
	1 750	–	66.9	53	92.1	57	Broadmeadow (1989a)

ppm: parts per million

The results of the PWG review of hepatocellular neoplasms indicate that there is an increased incidence of combined hepatocellular carcinoma and hepatocellular adenoma in male and female rats and male and female mice only at 5000 ppm acetochlor in the diet. This dose level greatly exceeds the MTD, as it produces hepatic and renal toxicity and is associated with a marked decrease in body weight and an increase in mortality. As an increased incidence of hepatocellular neoplasms occurred only at 5000 ppm, a dose that greatly exceeded the MTD in rats and mice, the use of these neoplasms for risk assessment is considered inappropriate (Hardisty, 1997a).

2.4 Genotoxicity

Results for gene mutation assays are conflicting and provide no clear evidence of a positive effect in either bacterial or mammalian cell test systems. Similarly, the evidence from in vitro and in vivo unscheduled DNA synthesis assays, in vitro sister chromatid exchange studies and an in vivo comet test provides no convincing pattern of genotoxic activity. By contrast, results from chromosomal aberration assays indicate that acetochlor is a confirmed clastogen in cultured human lymphocytes. There is also the possibility that the increased mutant colony counts observed in the positive mouse lymphoma assay resulted from a clastogenic rather than a mutagenic response, as this test system can detect chromosome breakage. Nevertheless, clastogenicity is confined to in vitro mammalian cell test systems, and the types of induced aberrations suggest cytotoxicity. Based on data from three bone marrow assays and three dominant lethal mutation studies in mice or rats, acetochlor-induced clastogenicity is not expressed in either somatic or germinal cells of whole animals. The results of studies of genotoxicity with acetochlor are summarized in Table 53.

Table 53. Results of studies of genotoxicity with acetochlor

End-point	Test object	Concentration	Purity (%)	Results	Reference
In vitro studies					
Bacterial gene mutation (Ames)	*Salmonella typhimurium* TA98, TA100, TA1535 and TA1537	0.001–1 µL/plate in ethanol (±S9)	92.5	Negative	Kulik & Ross (1978)
Bacterial gene mutation (Ames)	*S. typhimurium* TA98, TA100, TA1535, TA1537 and TA1538	1.6–5 000 µg/plate in DMSO (±S9)	89.9	Weakly positive[a]	Callander & Priestly (1989)
Bacterial gene mutation (Ames)	*S. typhimurium* TA1538	500–5 000 µg/plate in DMSO (±S9)	89.9 (technical standard) 99.6 (analytical standard)	Analytical-grade test material was negative Technical-grade test material was weakly positive	Callander (1992)
Bacterial gene mutation (Ames)	*S. typhimurium* TA1538	100–5 000 µg/plate in DMSO (±S9)	Technical acetochlor (89.9%, batch A) Technical acetochlor (94.4%, batch C) Analytical acetochlor (99.6%, batch B)	Negative[b]	Callander (1998)
Bacterial gene mutation (Ames)	*S. typhimurium* TA98, TA100, TA1535 and TA1537	33.3–5 000 µg/plate in DMSO (±S9)	95.5	Negative	Xu (2006a)
Bacterial gene mutation (Ames)	*S. typhimurium* TA98	33.3–5 000 µg/plate in DMSO (±S9)	95.5	Negative	Xu (2006b)
Gene mutation	CHO/HGPRT	25–150 µg/plate in ethanol (±S9)	96.3	Weakly positive	Li (1983)
Gene mutation	CHO/HGPRT	50–200 µg/mL in ethanol (±S9)	91.4	Negative	Li & Myers (1989)
Gene mutation	Mouse lymphoma	20–400 µL/L in DMSO (±S9)	Not available	Weakly positive	Mitchell, Rudd & Colman (1982)
Chromosomal aberration	Human lymphocytes	10, 50 and 100 µg/mL in DMSO (±S9)	89.4	Positive[c]	Howard (1989)
Chromosomal aberration	Human lymphocytes	10–150 µg/mL in DMSO (±S9)	99.6	Positive[d]	Fox (1998)

End-point	Test object	Concentration	Purity (%)	Results	Reference
Unscheduled DNA synthesis	Rat hepatocytes	0.016–160 µg/mL in DMSO (±S9)	99.7	Negative	Naismith (1983)
Sister chromatid exchange	Human lymphocytes	10 µmol/L in DMSO (±S9)	Not reported	Equivocal[e]	Hill et al. (1997)
In vivo studies: somatic cells					
Chromosomal aberration	Rat (bone marrow)	40–500 mg/kg bw in corn oil	96.3	Negative	Farrow & Cortina (1983)
Micronucleus	Mouse (bone marrow)	200–2 000 mg/kg bw in corn oil	96.7	Negative	Cavagnaro & Cortina (1985)
Micronucleus	Mouse (bone marrow)	898–1 719 mg/kg bw in corn oil	89.4	Negative	Randall (1989)
DNA damage: comet assay	Rat (nasal)	1750 ppm (87.5 mg/kg bw) in the diet	96.6	Negative[f]	Ashby et al. (1996)
Unscheduled DNA synthesis	Rat (hepatocyte)	500–2 000 mg/kg bw in corn oil	89.4	Weakly positive[g]	Trueman (1989)
Unscheduled DNA synthesis	Rat (hepatocyte)	500–2 000 mg/kg bw in corn oil	89.9	Weakly positive[h]	Ashby & Lefevre (1993, 1994)
In vivo studies: germ cells					
Dominant lethal	Rat	100–2 000 ppm in diet	94.3	Negative	Naylor (1987)
Dominant lethal	Rat	200–2 000 mg/kg bw in corn oil	90.4	Negative	Hodge (1991)
Dominant lethal	Rat	200–1 500 ppm in the diet	94.4	Negative	Milburn (1996a)
Dominant lethal	Mouse	200–3 500 ppm in the diet	94.4	Negative	Milburn (1996b)

bw: body weight; CHO: Chinese hamster ovary; DMSO: dimethyl sulfoxide; DNA: deoxyribonucleic acid; GSH: glutathione; HGPRT: hypoxanthine–guanine phosphoribosyltransferase; ppm: parts per million; S9: 9000 × *g* supernatant fraction from rat liver homogenate

[a] Weakly positive (< 2-fold increase) at higher concentrations in TA1538 (+S9). Negative in TA98, TA100, TA1535 and TA1537.

[b] Three different batches of technical-grade acetochlor were tested.

[c] Positive only at a concentration that induced substantial (36–69%) reduction in mitotic index.

[d] Positive response seen with acetochlor, but not with its *tert*-norchloroacetochlor (*t*-NCA; EU reference no. 6) analogue, indicating that the chloro substituent in acetochlor is responsible for its in vitro clastogenicity. Less toxicity observed in studies with whole blood than with isolated lymphocytes (probably due to protective effect of GSH).

[e] Equivocal (1.5-fold) increase in sister chromatid exchange at 10 µmol/L, maybe due to dialkylbenzoquinone metabolite in low-GSH environment.

[f] Negative at 1750 mg/kg bw per day, a dose level that produced rat nasal tumours.

[g] Weakly positive at 2000 mg/kg bw.

[h] Weakly positive at 2000 mg/kg bw, a dose level that also depleted hepatic GSH and produced severe liver toxicity.

2.5 Reproductive and developmental toxicity

(a) Multigeneration studies

Rats

In a two-generation reproductive toxicity study, acetochlor (purity 94.7%) was administered continuously in the diet to CD (SD) IGS BR (Sprague-Dawley) rats (26 of each sex per dose) at a nominal dose level of 0, 200, 600 or 1750 ppm (equal to 0, 18.6, 57.0 and 166 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). F_0 animals were given test article diet formulations for 10 weeks prior to mating to produce the F_1 litters. On PND 29, F_1 animals (26 of each sex per dose) were selected to become the parents of the F_2 generation and were given the same concentration of test formulation as their dams. F_1 animals were given test formulations for 10 weeks prior to mating to produce the F_2 litters.

The analytical data indicated that the mixing procedure was adequate and that variability was within acceptable ranges. No treatment-related clinical observations were observed in this study. Survival of parental animals was unaffected by treatment at any dose level. Mean premating body weight, body weight gain and feed consumption were consistently and statistically significantly reduced in both F_0 and F_1 animals at the high dose level (1750 ppm; Table 54). When compared with concurrent controls, decreases in body weight occurred in the high-dose F_0 males (reaching a maximum of approximately 8%) and females (reaching a maximum of 10%) from week 2 and throughout the premating period. The maximum decrease in F_1 animals was 16% for males and 14% for females. Body weights of F_1 females in the 600 ppm group were also decreased; the maximum difference from controls was approximately 9% in week 3. Body weights of F_0 and F_1 animals receiving 200 ppm acetochlor in the diet were similar to those of controls.

Table 54. Mean body weights of F_0 and F_1 parents

			Mean body weight (g)							
			Males				Females			
Generation	**Period**	**Weight**	**0 ppm**	**200 ppm**	**600 ppm**	**1 750 ppm**	**0 ppm**	**200 ppm**	**600 ppm**	**1 750 ppm**
F_0	Premating	Initial	153.5	155.8	150.9	150.9	126.9	122.7	122.5	123.3
		Final	463.6	463.2	465.5	433.5**	276.0	275.0	275.0	248.2**
	Gestation	Initial	–	–	–	–	284.3	276.0	273.4*	250.3*
		Final	–	–	–	–	380.5	385.4	378.5	365.4*
	Lactation	Initial	–	–	–	–	285.1	275.1*	271.5	248.4*
		Final	–	–	–	–	317.2	310.0	319.6	303.2*
F_1	Premating	Initial	76.3	80.2	74.8	67.5**	73.6	76.8	67.5**	64.9**
		Final	416.4	402.0	412.6	383.7**	251.5	251.1	246.9	229.8**
	Gestation	Initial	–	–	–	–	259.1	265.4	247.8	231.2**
		Final	–	–	–	–	353.6	354.9	351.6	345.1
	Lactation	Initial	–	–	–	–	257.4	271.9*	248.0	226.1**
		Final	–	–	–	–	298.9	301.7	297.5	294.9

ppm: parts per million; *: $P \leq 0.05$; **: $P \leq 0.01$
Source: Milburn (2001)

There was no effect of acetochlor on estrous cyclicity, precoital interval, the duration of gestation or number of successful matings. A decrease in mean number of implantations occurred in a

dose-related manner in both the F_0 and F_1 generations, reaching statistical significance at the high dose level in both generations. The mean number of live pups on PND 1 decreased in a dose-related manner in both the F_1 and F_2 litters. Mean live F_1 pups per litter was significantly decreased at 1750 ppm, and the mean number of live-plus-dead pups per litter was statistically significantly lower in F_1 and F_2 litters at 1750 ppm and also in F_2 litters at 600 ppm (Table 55).

Table 55. Effect of acetochlor on reproductive and offspring toxicity in F_0 and F_1 generations

	F_0				F_1			
Parameters	**0 ppm**	**200 ppm**	**600 ppm**	**1 750 ppm**	**0 ppm**	**200 ppm**	**600 ppm**	**1 750 ppm**
Estrous cycle (days)	3.89	3.86	3.80	3.70*	4.12	3.95	4.17	3.92
Precoital length (days)	2.27	2.60	2.65	2.58	2.80	3.33	2.88	3.48
Gestation length (days)	22.1	22.0	22.0	21.9**	22.0	21.9	21.9	21.8
Successful matings (%)	96.2	96.2	92.3	96.2	88.5	96.2	92.3	92.3
Whole litter loss (%)	16.0	8.0	12.5	12.0	17.4	8.0	8.3	8.3
No. of implantations	14.7	14.2	13.8	13.1**	14.6	13.6	13.5	12.7**
Postimplantation loss (%)	6.4	8.1	8.0	5.9	4.4	7.4	9.8*	7.7
No. of pups (live + dead)	13.7	13.0	12.6	12.3*	14.0	12.6	12.1*	11.7**
No. of live pups, day 1	13.1	12.3	12.1	12.0	13.6	12.3	11.9	11.4*

ppm: parts per million; *: $P \leq 0.05$; **: $P \leq 0.01$
Source: Milburn (2001)

There were no treatment-related gross pathological findings in the F_0 or F_1 adults. At 1750 ppm, relative kidney, liver and thyroid weights were significantly increased for F_0 and F_1 parental males and females, and relative ovarian weights were decreased for F_0 and F_1 females, compared with controls. Increased relative liver, kidney and thyroid weights were also observed at 600 ppm in F_0 males and females. However, there were no gross or histopathological changes in these tissues that appeared to be associated with treatment. Histopathological evaluation revealed treatment-related incidences of benign proliferative lesions (focal epithelial hyperplasia and polypoid adenomata [papillary adenoma]) in the epithelial lining of the ethmoid region of the nasal cavity in F_0 and F_1 adult animals receiving 1750 ppm acetochlor and in F_1 animals at the 600 ppm level (Table 56). Minimally increased brown pigment (lipofuscin) was observed in the olfactory mucosa, mainly in the lamina propria and occasionally in the basal epithelium, in most animals receiving 600 and 1750 ppm in both the F_0 and F_1 generations and also in F_1 females at the 200 ppm dose level; however, this finding was not considered to be of toxicological significance.

Table 56. Incidence of nasal proliferative lesions in F_0 and F_1 adults

		Males				Females			
Generation	**Findings**	**0 ppm**	**200 ppm**	**600 ppm**	**1 750 ppm**	**0 ppm**	**200 ppm**	**600 ppm**	**1 750 ppm**
F_0	*No. of tissues examined*	26	26	26	26	26	26	26	26
	Hyperplasia	0	0	0	3	0	0	0	7
	Papillary adenoma	0	0	0	4	0	0	0	6
F_1	*No. of tissues examined*	26	26	26	26	26	26	26	26
	Hyperplasia	0	0	0	7	0	0	4	14
	Papillary adenoma	0	0	3	8	0	0	1	17

ppm: parts per million
Source: Milburn (2001)

Postnatal survival was not affected by treatment. The adjusted body weights of the pups at preweaning were statistically significantly reduced in the 1750 ppm groups for the F_1 and F_2 generations and in the 600 ppm group for the F_2 generation. There was no effect on body weights of F_1 or F_2 pups in the 200 ppm group. There was no effect of acetochlor on preputial separation in F_1 males. A decrease in anogenital distance in F_2 males on PND 1 and a 3-day treatment-related delay in the day of vaginal opening in F_1 females at the high dose level appeared to be associated with delayed growth. The delay in vaginal opening was attributed to the delay in reaching critical body weight and was not considered to be a specific developmental effect, as the body weight at the time of vaginal opening was similar to that of controls. There was no effect of treatment on vaginal opening in F_1 females in the 200 and 600 ppm groups (Table 57). Sperm measures (epididymal sperm count, motility and morphology; and testicular spermatid count) were not affected by treatment for F_0 or F_1 males. There were no treatment-related histopathological lesions of the reproductive organs, and primordial follicle count data were similar between control and high-dose F_1 females.

Table 57. Effect of acetochlor on developmental parameters of F_1 and F_2 pups

	F_1				F_2			
Developmental parameters	**0 ppm**	**200 ppm**	**600 ppm**	**1 750 ppm**	**0 ppm**	**200 ppm**	**600 ppm**	**1 750 ppm**
Pup survival (%), day 29	74.7	87.0	86.5	91.0**	90.5	88.4	92.3	92.9
Sex distribution (% male)	49.6	49.7	51.6	47.5	50.2	51.4	54.3	54.7
Pup weight (g), day 29, M	6.0	6.0	5.7*	5.8*	5.7	5.9	5.9	5.5
Pup weight (g), day 29, F	5.7	5.7	5.3	5.3*	5.4	5.6	5.5	5.2
Pup weight (g), day 29, M[a]	76.8	80.9	75.8	69.2*	75.8	73.7	68.3**	65.0**
Pup weight (g), day 29, F[a]	72.6	74.5	70.4	65.1*	71.9	70.3	64.4**	61.0**
Preputial separation (days)	46.9	46.8	47.0	48.0	–	–	–	–
Vaginal opening (days)	36.2	35.8	37.8	39.1**	–	–	–	–
Body weight at vaginal opening (g)	112.9	113.5	110.4	109.4	–	–	–	–
Anogenital distance (mm), M	–	–	–	–	4.49	4.50	4.45	4.32*
Anogenital distance (mm), F	–	–	–	–	2.66	2.72	2.70	2.64

F: female; M: male; ppm: parts per million; *: $P \leq 0.05$; **: $P \leq 0.01$

[a] Adjusted by covariance on day 1 mean pup weight.

Source: Milburn (2001)

There were no treatment-related macroscopic findings in either sex of the pups at any dose level. Decreases in absolute mean brain weights of F_1 males and F_2 males and females were noted at 1750 ppm. On a relative to body weight basis, ratios were statistically increased for the brain in F_2 pups at the 600 ppm dose as well. Mean absolute spleen weights were also decreased in F_1 males and in both sexes of the F_2 pups at both the middle and high dose levels. Spleen weights were also reduced on a relative basis in 600 ppm F_2 females. Mean absolute thymus weights were also decreased in F_2 pups at the high dose level, but not relative (to body weight) thymus weights.

The NOAEL for parental toxicity was 200 ppm (equal to 18.6 mg/kg bw per day), based on focal hyperplasia and polypoid adenomata in the nasal epithelium of adult F_1 offspring at study termination and decreased body weight in F_1 females observed at 600 ppm (equal to 57.0 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 seen 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 observed at 600 ppm (equal to 57.0 mg/kg bw per day) (Milburn, 2001).

In a second two-generation reproductive toxicity study, acetochlor (purity 91.0%) was administered continuously in the diet to CD (Sprague-Dawley) rats (25 of each sex per dose) at a nominal dose level 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). F_0 animals were given test article diet formulations for 8 weeks prior to mating to produce the F_1 litters. On postnatal day (PND) 29, F_1 animals (26 of each sex per dose) were selected to become the F_1 parents of the F_2 generation and were given the same concentration of test formulation as their dams. F_1 animals were given test formulations for 10 weeks prior to mating to produce the F_2 litters. Animals were dosed 40 days prior to mating. Quantitative evaluation of primordial follicles, sperm parameters, vaginal opening, balano-preputial separation and anogenital distance was not conducted.

The analytical data indicated that the mixing procedure was adequate and that variability was within acceptable ranges. No compound-related effects on clinical signs of toxicity or mortality were observed. The body weight gain of animals receiving 1750 ppm acetochlor was reduced compared with that of controls during maturation in both generations, although there was little effect on females during the gestation or lactation phases. Body weight gain of animals receiving 18 or 175 ppm acetochlor was similar to that of controls. The comparison of the mean parental body weights is shown in Table 58.

Feed consumption by males in both generations at 1750 ppm was slightly reduced compared with controls, but there was no effect in females at this dose or in either sex at 18 or 175 ppm acetochlor. The mating performance, conception and fertility indices were similar in all groups at both pairings. The precoital interval was very similar between groups (1–4 days) for the majority of animals in the F_0 (92% and 100% in control and 1750 ppm groups, respectively) and F_1 generations (92% and 84% in control and high-dose groups, respectively). There were no treatment-related macroscopic or histopathological findings in either generation. Organ weights (brain, liver and kidney in both sexes, heart and reproductive organs in F_1 males, and spleen in females) relative to body weight were increased for animals in the 1750 ppm group as a result of the considerably lower final body weights of these animals compared with controls. Thymus weight was reduced in the F_1 males. In addition, there was an increase in the relative weights of epididymides, testes, seminal vesicles and thymus in F_1 males.

There was no evidence of an adverse effect of acetochlor on pup birth weight, pup sex ratio, pup viability or pup development. At the end of the lactation period, the body weight of the pups of both generations at the 1750 ppm dose level was reduced compared with controls (14–20% lower than controls). Macroscopic examination of F_1 and F_2 pups did not reveal any treatment-related abnormalities.

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 in the premating period as well as body weight reduction at the beginning of the gestation and lactation periods and, at sacrifice, 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 observed 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 (Willoughby, 1989).

In a third two-generation reproductive toxicity study, acetochlor (purity 94.5%) was administered continuously in the diet to COBS CD rats (12 males and 24 females per dose) at a nominal dose level 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 442 mg/kg bw per day for F_0 females, respectively). F_0 animals were given test article diet formulations for 100 days and F_1 parents for 120 days prior to mating. Data were

Table 58. Mean body weights of F_0 and F_1 parents in a reproductive toxicity study in rats

			Mean body weight (g)							
			Males				Females			
F	Period	Weight	0 ppm	18 ppm	175 ppm	1 750 ppm	0 ppm	18 ppm	175 ppm	1 750 ppm
F_0	Premating	Initial	252	252	248	251	170	169	170	173
		Final	494	493	488	469**	283	272	279	265**
	Gestation, F_{1A}	Initial	–	–	–	–	284	274	276	261**
		Final	–	–	–	–	396	392	395	374
	Lactation, F_{1A}	Initial	–	–	–	–	306	304	307	275**
		Final	–	–	–	–	327	327	322	304
	Gestation, F_{1B}	Initial	–	–	–	–	318	309	309	284**
		Final	–	–	–	–	439	434	434	397
	Lactation, F_{1B}	Initial	–	–	–	–	340	329	328	291**
		Final	–	–	–	–	356	357	354	323
F_1	Premating	Initial	79	81	77	75	70	74	70	67
		Final	532	522	530	472**	279	276	285	246**
	Gestation, F_{2A}	Initial	–	–	–	–	283	275	285	247**
		Final	–	–	–	–	387	391*	401*	348
	Lactation, F_{2A}	Initial	–	–	–	–	316	301	314	262**
		Final	–	–	–	–	324	323	322	281
	Gestation, F_{2B}	Initial	–	–	–	–	317	312	321	263**
		Final	–	–	–	–	430	432	441	363*
	Lactation, F_{2B}	Initial	–	–	–	–	353	346	357	286**
		Final	–	–	–	–	340	352	357	296

ppm: parts per million; *: $P \leq 0.05$; **: $P \leq 0.01$ (analysis of variance and Student's *t*-test)
Source: Willoughby (1989)

lacking on the evaluation of precoital interval, primordial and small growing follicles, mean number of cycles and cycle length, number of implantations, whole litter losses, developmental landmarks and sex ratios. Postmortem examinations and histopathology were limited to those animals that died on test and five F_{1b} and F_{2b} pups and 10 F_1 parents killed at scheduled sacrifice.

There were no treatment-related effects on survival, clinical observations, fertility indices, sperm parameters, gestation length, gross pathology or organ weights. At termination, the mean body weights of F_0 animals at 5000 ppm were statistically significantly decreased by 10.4% (male) and 25.1% (female) compared with controls. Similarly, the mean body weights of F_1 animals at 5000 ppm were statistically significantly decreased by 19.1% (male) and 33.4% (female) compared with controls. At 1500 ppm, body weights of females were statistically significantly decreased (11.4%) compared with controls. At the 5000 ppm level, feed consumption was reduced by 24% and 27% in females of the F_0 and F_1 generations, respectively. At 5000 ppm, the absolute weights of the heart, brain and spleen were decreased in both sexes. As well, the relative weights of kidney and liver were increased at 5000 ppm; relative liver weight was also increased in both sexes in the F_1 generation at 1500 ppm. In females, the relative weight of adrenals decreased and the absolute and relative weights of the thyroid increased at 5000 ppm. In males, the absolute weight of the pituitary decreased at 500 and 5000 ppm, and the relative weight of the pituitary decreased at 500 and 1500 ppm. A slight

increase was observed in the relative weight of adrenals at 5000 ppm. The only histopathological finding in the parent animals was nephritis in 8/10 females of the F_1 generation at 5000 ppm.

There were no effects of acetochlor on the clinical condition of the F_1 or F_2 pups. Statistically significant differences were observed in the number of live pups and the mean body weight of pups (37–45% compared with controls) from the F_1 and F_2 generations at 5000 ppm. At 1500 ppm, there was a slight, but statistically significant, decrease in the body weight of F_{2b} pups at lactation days 14 and 21. There were no test article–related changes in either mean absolute or mean relative (to body weight) organ weights observed among animals killed at the scheduled sacrifice in F_1 and F_2 animals, except for spleen weights in F_{2b} pups at 5000 ppm.

In conclusion, the NOAEL for parental systemic toxicity was 500 ppm (equal to 30.8 mg/kg bw per day), based on decreased body weights and decreased relative weights of liver and kidney observed 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 the number of liveborn pups and decreased body weights of F_{2b} pups observed 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) (Schardein, 1982).

(b) Developmental toxicity

Rats

In a prenatal developmental toxicity study, acetochlor (purity 90.5%) was administered by gavage in corn oil to 25 pregnant Sprague-Dawley CD (SD) BR VAF/Plus rats per dose from gestation day (GD) 6 to GD 15 inclusive at a dose level of 0, 40, 150 or 600 mg/kg bw per day. Animals were killed on GD 20, and uteri were examined for live fetuses and intrauterine deaths. Fetuses were weighed and examined for external, visceral and skeletal alterations.

Four animals were killed moribund. Two females dosed with 600 mg/kg bw per day were killed (one on GD 13 and the other one on GD 15) with signs including piloerection, hunched posture, weight loss and general poor condition. Two further females, one control and one dosed with 40 mg/kg bw per day, revealed perforated oesophagus, indicating an intubation error. There was a dose-related response in the extent of salivation observed post-dosing (15, 25 and 23 of the rats dosed at 40, 150 and 600 mg/kg bw per day, respectively). Signs of toxicity were generally confined to the 600 mg/kg bw per day group and included ungroomed coat, piloerection and hunched posture in two females and stained urogenital region in four females. Rats dosed with 600 mg/kg bw per day reduced their feed consumption slightly, but statistically significantly, during days 6–9 (about 20%); thereafter, feed intake was slightly lower than, but comparable with, that of controls. A statistically significant increase in water consumption (28.5–52.4% increase compared with controls) was observed during GDs 6–15 at 600 mg/kg bw per day. At 600 mg/kg bw per day, the rate of body weight gain when compared with the control group was statistically significantly reduced ($P < 0.01$) from the first dose (GD 6), which was followed by a statistically significant mean weight loss from GD 7 to GD 9 (about 10.6% compared with controls). The mean rate of weight gain thereafter was still slightly lower than that of controls through to GD 12, although the difference from control weight gain was not statistically significant. From day 13, body weight gains were comparable with those of the control group, although parity was not regained by GD 20. Macroscopic findings at postmortem examination did not reveal any treatment-related effects.

Only at the 600 mg/kg bw per day dose level was there a statistically significant increase in early embryonic deaths, total embryonic deaths and postimplantation loss. However, the increases observed were predominantly due to a higher number of litters with one embryonic death at this dose and do not appear to be directly attributable to treatment; the number of litters with more than one embryonic death were one, four, two and two at 0, 40, 150 and 600 mg/kg bw per day, respectively. A statistically significant decrease in mean fetal weight was also observed at the high dose (Table 59).

Table 59. Selected gestational and fetal parameters in a developmental toxicity study in rats

Parameter	0 mg/kg bw per day	40 mg/kg bw per day	150 mg/kg bw per day	600 mg/kg bw per day
Early embryonic deaths	0.2	0.5	0.5	0.8**
Late embryonic deaths	0.1	0	0	0.2
Total embryonic deaths	0.3	0.5	0.5	1.0**
Postimplantation loss (%)	3.4	4.4	4.3	7.6**
Mean fetal weight (g)	3.37	3.32	3.36	3.13**

bw: body weight; **: $P < 0.01$
Source: Brooker, Stubbs & John (1989b)

Some reduction in ossification was also observed at the highest dose level. There was no obvious relationship between treatment and the incidence or distribution of malformations (three, three, six and four malformed fetuses at 0, 40, 150 and 600 mg/kg bw per day, respectively) or visceral anomalies.

The NOAEL for maternal toxicity in rats was 150 mg/kg bw per day, based on mortality, clinical signs of toxicity, decreases in 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 the reduction in mean fetal weight and reduced ossification at 600 mg/kg bw per day. There was no evidence of teratogenicity (Brooker, Stubbs & John, 1989b).

In a second prenatal developmental toxicity study, acetochlor (purity 91.3%) was administered by gavage in corn oil to 25 pregnant Sprague-Dawley COBS CD rats per dose from GD 6 to GD 19 inclusive at a dose level of 0, 50, 200 or 400 mg/kg bw per day. The corn oil gavage dosing volume was 10 mL/kg bw. Caesarean sections were performed on all dams on GD 20, and uteri were examined for live fetuses and intrauterine deaths. Fetuses were weighed and examined for external, visceral and skeletal alterations.

Mortality was not observed in the study. Matting and/or staining of the anogenital region were noted for 13/25 rats in the 400 mg/kg bw per day group, and excessive salivation was observed in three rats as a post-dosing response on one occasion. A slight but not dose-related increase in matting and/or staining of the anogenital region was noted in the 50 and 200 mg/kg bw per day dose groups. At 400 mg/kg bw per day, decreases in body weight gain (30.4% of the control value) and adjusted body weight gain (53.5% of the control value) were noted for the GDs 6–20 and GDs 0–20 periods, respectively. No significant differences in mean maternal body weight gain during the treatment period or adjusted body weights on GD 20 were observed in the 50 or 200 mg/kg bw per day dose groups when compared with the control group.

There were no biologically meaningful or statistically significant differences in the mean number of corpora lutea, total implantations, postimplantation loss, viable fetuses or the fetal sex distribution in the 50, 200 or 400 mg/kg bw per day dose groups when compared with the control group. A slight to moderate decrease in mean fetal weight, although not statistically significant, was noted in the 400 mg/kg bw per day dose group. There were no statistically significant differences in the number of litters with malformations in the 50, 200 or 400 mg/kg bw per day dose groups when compared with the control group. The only malformation noted in the 400 mg/kg bw per day dose group was dwarfism, noted in five fetuses in one litter (1.9% incidence). The incidence of dwarfism fell into the range of the historical control data presented (0–1.9% incidence in fetuses and 0–4.3% incidence in litters).

Based on the results of this developmental toxicity study in rats, the NOAEL for maternal toxicity was 200 mg/kg bw per day, based on the decreased body weight gain 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. There was no evidence of teratogenicity in rats (Rodwell, 1980).

Rabbits

In a developmental toxicity study, acetochlor (purity 90.5%) was administered by gavage in corn oil to 16 pregnant New Zealand White rabbits per dose from GD 6 to GD 18 inclusive at a dose level of 0, 30, 100 or 300 mg/kg bw per day. Caesarean sections were performed on all dams on GD 29, and uteri were examined for live fetuses and intrauterine deaths. Fetuses were weighed and examined for external, visceral and skeletal alterations.

At 300 mg/kg bw per day, one female was killed following an intubation error; a second female was unable to move easily, with restricted movement of the hindlimbs (the toxicological significance of this is unclear), and was killed on GD 11; and a third female was found dead on GD 19 after aborting on GD 18. No clinical signs of toxicity were seen that were considered to be due to treatment in any group. There was a statistically significant decrease in body weight gain (Table 60) during GDs 6–8 at 300 mg/kg bw per day (weight change of −30, −21, −20 and −83 g at 0, 30, 100 and 300 mg/kg bw per day, respectively). Body weight gain adjusted for gravid uterine weight did not show any intergroup differences. At 300 mg/kg bw per day, there was a statistically significant reduction in feed consumption during GDs 6–7 (mean feed consumption of 125, 126, 120 and 86 g per rabbit per day at 0, 30, 100 and 300 mg/kg bw per day, respectively). Macroscopic findings at postmortem examination did not reveal any treatment-related effects.

Table 60. Body weight change in dams in a developmental toxicity study in rabbits

	Body weight change (g)			
Days of gestation	**0 mg/kg bw per day**	**30 mg/kg bw per day**	**100 mg/kg bw per day**	**300 mg/kg bw per day[a]**
1–6	59	24	6	47
6–8	−30	−21	−20	−83*
8–11	−2	4	33	39
11–15	102	136	107	145
15–18	15	8	16	−13
18–25	166	126	100	137
25–29	144	75	158	110

bw: body weight; *: $P < 0.05$

[a] Ten animals.

Source: Brooker, Stubbs & John (1989a)

There were no treatment-related effects of acetochlor on litter size, sex ratio, preimplantation and postimplantation losses, litter weight, mean fetal weight or incidence of malformations, visceral and skeletal anomalies or skeletal variants at any dose level.

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 treatment-related death of two dams observed 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 (Brooker, Stubbs & John, 1989a).

In a second developmental toxicity study, acetochlor (purity 94.2%) was administered by gavage in corn oil to 20 inseminated New Zealand White rabbits per dose from GD 7 to GD 19 inclusive at a dose level of 0, 15, 50 or 190 mg/kg bw per day. Animals were killed on GD 29, and

uteri were examined for live fetuses and intrauterine deaths. Fetuses were weighed and examined for external, visceral and skeletal alterations. Feed consumption was not recorded, gravid uterine weights were not determined, and dosing was only on GDs 7–19.

There were no treatment-related effects on survival, clinical observations or gestational parameters. Statistically significant decreases in mean body weight on GD 19 (3748 g treated mean versus 4038 g in controls) and mean body weight loss on GDs 7–19 (−146 g in treated versus +81 g in controls) were observed at 190 mg/kg bw per day. Macroscopic findings at postmortem examination did not reveal any treatment-related effects.

Intrauterine survival, fetal sex ratios and fetal weights were not affected by administration of the test article at any of the dose levels. No remarkable differences in the number or type of malformations were apparent in any of the treated groups when compared with the vehicle control group. Fetal developmental variations occurred with similar frequency in the control and treated groups.

The NOAEL for maternal toxicity was 50 mg/kg bw per day, based on decreased body weight at GD 19 and decreased body weight gain during GDs 7–19 observed 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 (Adam, 1986).

2.6 Special studies

(a) Neurotoxicity

Rats

In an acute neurotoxicity study, groups of fasted Alpk:ApfSD (Wistar-derived) rats (10 of each sex per dose) were given a single oral dose of acetochlor (purity 94.7%) in corn oil at a dose of 0, 150, 500 or 1500 mg/kg bw and observed for 14 days. Neurobehavioural assessment (functional observational battery and motor activity testing) was performed in 10 animals of each sex per group pretest and at study days 1 (time of peak effect), 8 and 15. At study termination, five animals of each sex per group were euthanized and perfused in situ for neuropathological examination. Of the perfused animals, five rats of each sex from the control and high-dose groups were subjected to histopathological evaluation of brain and peripheral nervous system tissues.

One male given 1500 mg/kg bw was found dead on day 2. This death is attributed to the acute toxicity of acetochlor. At 1500 mg/kg bw, body weights adjusted for initial weight were significantly lower than in the control group on day 8 for males and on days 1, 8 and 15 for females. Feed consumption by the high-dose males and females was significantly reduced during the first week of the study compared with the controls. During the functional observational battery, findings were limited to the time of peak effect at the high dose level. At 1500 mg/kg bw, the following clinical signs were attributable to treatment: decreased activity, chromodacryorrhoea, hunched posture, hypothermia, laboured breathing, piloerection, sides pinched in, signs of salivation, stains around the mouth and nose and upward curvature of the spine. Almost all animals recovered by day 8, but a few presented with slightly reduced splay reflex on day 15. In male and female rats given 500 mg/kg bw, the clinical signs were piloerection and stains around the nose on day 1 only. No effects of treatment on landing foot splay measurement, time to tail flick or grip strength were noted.

No effects of treatment on motor activity were noted in males (Table 61). Total activity counts for high-dose females on day 1 were significantly decreased at 1500 mg/kg bw compared with controls. In females given 500 mg/kg bw, motor activity on day 1 was statistically significantly decreased relative to controls. However, the motor activity level in the 500 mg/kg bw dose group on day 1 was slightly greater than the pretest value. Furthermore, the pattern of habituation was unchanged compared with pretest values. Consequently, this decrease is not considered to be related to treatment with acetochlor. A statistically significant increase in motor activity of 28% was also observed in high-dose females on day 8. There were no treatment-related effects on brain weights or gross and histological pathology or neuropathology. Effects on the motor activity and functional

observational battery parameters at the high dose of 1500 mg/kg bw are considered to be due to the overt toxicity of the compound rather than neurotoxicity.

Table 61. Intergroup comparison of motor activity (total activity and percentage of pretest values)

	Males				Females			
Period (days)	**0 mg/kg bw**	**150 mg/kg bw**	**500 mg/kg bw**	**1 500 mg/kg bw**	**0 mg/kg bw**	**150 mg/kg bw**	**500 mg/kg bw**	**1 500 mg/kg bw**
−7	398.5	390.3	436.9	437.6	423.6	392.7	345.5	448.1
1	361.2	600.5	388.8	269.2	571.4	501.4	401.7**	251.7**
% of pretest value	90.6	77.0	88.6	61.5	134.9	127.7	116.3	56.2
8	566.6	581.6	487.0	575.6	525.8	570.9	573.0	673.2*
% of pretest value	142.2	149.0	111.0	131.5	124.1	145.4	165.8	150.2
15	516.3	538.1	609.4	504.2	622.6	587.3	632.3	642.2
% of pretest value	129.6	137.9	138.8	115.2	147.0	149.6	183.0	143.3

bw: body weight; *: $P < 0.05$; **: $P < 0.01$ (analysis of variance)
Source: Kilgour (2001a)

The NOAEL for systemic toxicity was 500 mg/kg bw, based on decreased body weight and body weight gain and reduced feed consumption in both sexes observed at 1500 mg/kg bw. The NOAEL for neurotoxicity was 1500 mg/kg bw, the highest dose tested (Kilgour, 2001a).

In a subchronic oral neurotoxicity study, acetochlor (purity 94.7%) was administered in the diet to 12 Alpk:APfSD rats of each sex per group at a dose level of 0, 200, 600 or 1750 ppm (equal to 0, 15.4, 47.6 and 139 mg/kg bw per day for males and 0, 18.3, 55.9 and 166.5 mg/kg bw per day for females, respectively) for 90 days. A neurobehavioural assessment (functional observational battery and motor activity testing) was performed in all animals of each sex per group at −1 week pretest and at weeks 2, 5, 9 and 14. At study termination, five animals of each sex per group were euthanized and perfused in situ for neuropathological examination (brain, spinal cord and peripheral nervous system of the control and high-dose animals were examined microscopically; brain weights were also measured).

At 1750 ppm, slight but statistically significant decreases in mean body weight (−2.6% to −4.1% less than controls) and weight gain (−14% to −20% for males and −25% to −30% for females) were reported in both sexes in the early weeks of the study. Decreases thereafter were not statistically significant, but continued throughout the study. Body weight gain was also lower in animals given 1750 ppm acetochlor in the diet. The maximum effect was seen in week 2. During the functional observational battery evaluations at week 2, but not at later times, a statistically significant decrease in hindlimb grip strength (−44%) in males was observed, which was considered to be a transient effect, as it was not observed at any other time points. There were no treatment-related increases in clinical signs of toxicity or effects on other neurobehavioural parameters in the functional observational battery, motor activity, brain weight or gross/microscopic neuropathology.

The NOAEL for systemic toxicity was 600 ppm (equal to 47.6 mg/kg bw per day), based on marginal decreases in mean body weight and body weight gain in males and females observed at 1750 ppm (equal to 139 mg/kg bw per day) (Kilgour, 2001b).

(b) Immunotoxicity

Mice

In an immunotoxicity study, acetochlor (purity 95.4%) was administered to 10 female Crl:CD1(ICR) mice per dose 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. A concurrent positive control group received cyclophosphamide monohydrate at 50 mg/kg bw per day once daily during study days 24–27 via intraperitoneal injection. Only female animals were used for the immunotoxicity evaluation because females are considered to be more consistent responders than males for the evaluation of the humoral immunoresponse using the antibody-forming cell (AFC) assay. All animals were immunized with an intravenous injection of sheep red blood cell antigen in the tail vein on study day 24.

Over the entire 4-week study period, there were no test substance–related effects on survival, body weight, feed consumption or parameters evaluated during histopathological examinations. There were no test substance–related effects on absolute or relative (to final body weight) spleen or thymus weights, spleen cellularity or the T cell–dependent antibody response, as measured by the AFC immunoglobulin M specific activity (AFC/10^6 spleen cells) or total spleen activity (AFC/spleen), at any dose level tested.

The NOAEL for immunotoxicity was 5000 ppm (equal to 1536 mg/kg bw per day), the highest dose tested (Crittenden, 2011).

(c) Mechanistic studies on nasal tumours

In vivo studies

A study was designed to determine the overall tissue distribution and localization, especially in the nasal turbinate tissue, of ^{14}C-labelled acetochlor residues following oral administration to rats and mice. The nasal turbinate is a target tissue in rats.

The study consisted of two groups of animals. In the first group, three male and three female Sprague-Dawley rats were administered a single oral gavage dose of ^{14}C-labelled acetochlor (purity 99.5%) at target dose levels of 290 and 375 mg/kg bw for males and females, respectively. The second group consisted of three male and three female B6C3F1 mice administered a single oral gavage dose of ^{14}C-labelled acetochlor at target dose levels of 885 and 1060 mg/kg bw for males and females, respectively. These dose levels correspond to the high dose levels used in the rat and mouse carcinogenicity studies described above in section 2.3 (Ahmed, 1983a,b; Naylor & Ribelin, 1986; Amyes, 1989; Broadmeadow, 1989a). For both mice and rats, one animal of each sex was killed at 6 and 24 hours and 7 days after dosing. All animals were processed by whole-body autoradiography.

In the male rat killed at 6 hours after dosing, intense, nonspecific localization was seen in the frontal region of the nose. Specific localization was also observed in the ethmoid turbinate region. Because of the unusual nature of the nonspecific localization, the 6-hour time point was repeated for both male and female rats. The nonspecific nasal localization was not repeated; however, localization was observed in the ethmoid turbinates of both male and female rats. Few differences were observed in the sites of localization between males and females. Overall, the sites of distribution of radioactivity in the body were similar in the male and female rats. At 7 days after treatment, there was still slight radioactivity present in nasal tissue, blood and highly perfused tissues. At both 24 hours and 7 days, females generally displayed a slightly higher accumulation of radioactivity compared with males.

No evidence of nasal localization of radioactivity was observed in either the male or female mice at any time point. At 7 days, most of the radioactivity appeared to have been cleared from the tissues in male mice, but not in female mice.

Acetochlor and/or its metabolites were found to accumulate specifically in the olfactory region of the nasal turbinates in the rat and remained detectable for at least 7 days. No specific accumulation of acetochlor and/or its metabolites was observed in the olfactory region of the nose in mice (Kraus & Wilson, 1996).

A study was conducted to evaluate the nasal localization of ^{14}C-labelled acetochlor following dietary administration to rats. A mixture of radiolabelled (^{14}C) and unlabelled (^{12}C) acetochlor was administered in the diet at target levels of 1750 and 5000 ppm for 14 days to Sprague-Dawley rats. Three males per group were used in groups M1 and M2, and four males per group were used in groups M3 and M4. Groups M1 and M3 received 1750 ppm acetochlor, and groups M2 and M4 received 5000 ppm acetochlor. Animals in groups M1 and M2 were used in the characterization of nasal protein adducts, whereas those in groups M3 and M4 were used for whole-body autoradiography and nasal turbinate microautoradiography studies.

Results from whole-body autoradiography showed significant localization of radioactivity in nasal turbinates. Localization of radioactivity was apparent at both 1750 and 5000 ppm. Microautoradiography studies showed intense localization of radioactivity in the Bowman's glands of the group M4 animals. A lower degree of localization of radioactivity in the olfactory surface epithelium was observed, whereas no evidence of localization was evident in the respiratory epithelium. For group M3 animals, only slight to moderate localization of radioactivity was observed in the Bowman's glands of the animals. This study demonstrated the formation of an acetochlor protein adduct in rat nasal tissue that is derived from the quinoneimine, 3-ethyl,5-methylbenzoquinone-4-imine (EMIQ).

This result supports the hypothesis that rat nasal tissue is capable of metabolizing acetochlor to EMIQ. The formation of EMIQ in rat nasal tissue could be a critical step for the induction of nasal tumours in rats by acetochlor (Lau et al., 1998a).

A study was conducted to determine and characterize the nasal localization of radiolabel in Sprague-Dawley rats following oral administration of ^{14}C-labelled acetochlor secondary sulfide. A mixture containing radiolabelled (^{14}C) acetochlor sulfide and unlabelled (^{12}C) acetochlor was administered by oral gavage to four groups of male Sprague-Dawley rats at approximately 7 mg/kg bw. Groups M1, M2 and M3 were dosed for 5 consecutive days, and group M4 received a single dose. Two out of four rats from group M1 and one out of two rats from group M2 were killed 5 days after the final dose; all other rats were killed 1 day after their final/single dose. Groups M1, M2 and M4 were used for whole-body autoradiography or microautoradiography, and group M3 was used for characterization of binding to nasal tissue.

HPLC analysis of the acid hydrolysate of rat nasal proteins from rats treated with ^{14}C-labelled acetochlor secondary sulfide showed a major radioactive component in the acid hydrolysate that was structurally related to 3-ethyl,5-methylbenzoquinoneimine-cysteine (EMIQ-cysteine). It was tentatively assigned as the L-cysteine conjugate of EMIQ-(4-amino-2-(cysteine-*S*-yl)-2-ethyl,5-methylphenol. Unlike the results obtained from rats administered ^{14}C-labelled acetochlor, significant amounts of EMA were not observed in the hydrolysate of nasal proteins. The lack of formation of EMA in the acid hydrolysate of acetochlor secondary sulfide–treated animals provides evidence that the chlorine displacement of acetochlor by the protein sulfhydryl was a potential pathway in the binding of acetochlor to rat nasal proteins. Results from whole-body autoradiography showed significant localization and persistence of radioactivity in nasal turbinates. Microautoradiography studies showed intense localization of radioactivity in the Bowman's glands of treated animals.

In conclusion, the formation of an EMIQ-derived protein adduct in rat nasal turbinate after oral treatment with acetochlor secondary sulfide has been reported. As acetochlor secondary sulfide lacks the chlorine atom in its amide side-chain, the binding of sulfhydryl in protein via the chlorine displacement route is not feasible. This is supported by the lack of formation of EMA in the acid hydrolysate of rat nasal turbinate. Autoradiography showed the localization of radioactivity in rat nasal turbinates after oral administration of acetochlor secondary sulfide. This finding is consistent with the hypothesis that acetochlor secondary sulfide is metabolized to the precursor for EMIQ, which binds to protein covalently in the rat nasal turbinate (Lau et al., 1998b).

In a distribution study, groups of Crl:CD(SD)BR rats were given a single oral gavage dose (200 mg/kg bw) of ^{14}C-ring-labelled *sec*-amide methyl sulfoxide (EU reference no. 46; also known as acetochlor sulfoxide; purity > 99%), ^{14}C-carbonyl-labelled acetochlor sulfoxide (purity > 99%) or ^{14}C-phenyl ring–labelled EMA (EU reference no. 34; purity > 97.0%) in polyethylene glycol 600. Nasal tumours were seen in rats, but not in mice. The target specificity in the rat is believed to be due to the metabolism of acetochlor to products such as quinoneimines, which bind covalently to nasal proteins. Acetochlor sulfoxide (EU reference no. 46), which is the major plasma metabolite in rat metabolism studies, has been found to specifically accumulate in the olfactory region of rat nasal tissue. The acetochlor sulfoxide (EU reference no. 46) was metabolized to EMA (EU reference no. 34), which is an intermediate precursor to EMIQ. Two rats from each dose group were killed at 8 and 24 hours and 5 days after dosing. A 2% (w/v) solution of EMA in carboxymethyl cellulose was injected into the external nasal passages, and the carcasses were immediately frozen in hexane/dry ice. The rats were then processed for autoradiography.

Most of the radioactivity of ^{14}C-ring-labelled acetochlor sulfoxide (EU reference no. 46) showed selective labelling in the olfactory region of nasal passages at all of the time points, including 5 days after dosing. At 8 and 24 hours, the radioactivity was concentrated in the olfactory region of the nasal passages and the gastrointestinal tract, and at 5 days, the only radioactivity present in the animal was in the nasal turbinates. ^{14}C-carbonyl-labelled acetochlor sulfoxide (EU reference no. 46) also showed a similar pattern of labelling, with localization in the olfactory region of the nasal passages. ^{14}C-phenyl ring–labelled EMA (EU reference no. 34) also localized in the nasal passages, although this metabolite did not show the olfactory-specific localization seen with acetochlor sulfoxide (EU reference no. 46). Radiolabel was present throughout the nasal passages, in the lining of the buccal cavity and in the nares.

In conclusion, the persistence of acetochlor sulfoxide (EU reference no. 46), the major metabolite circulating in plasma, in the olfactory region of the nasal passage 5 days after dosing strongly suggests that the radioactivity was covalently bound to protein. EMA (EU reference no. 34) also localized in the nasal passages but did not appear to show the olfactory specificity of the acetochlor sulfoxide (Green, 1997a).

A comparative dietary feeding study was conducted to assess the carcinogenic potential of *sec*-amide methyl sulfoxide (also known as acetochlor sulfoxide; EU reference no. 46), a metabolite of acetochlor, to the nasal passages of male CD (Sprague-Dawley) rats. Three groups of 96 rats were fed ad libitum on control diet, diet containing 1750 ppm acetochlor (purity 94.7%) or diet containing acetochlor sulfoxide (purity 96%). The initial dietary concentration of acetochlor sulfoxide (100 ppm) was based on preliminary studies that showed that 100 ppm acetochlor sulfoxide gave plasma sulfoxide levels comparable to those resulting from the 1750 ppm diet selected for acetochlor. Chemical consumption was on average 14.6 mg/kg bw per day for acetochlor sulfoxide and 99.6 mg/kg bw per day for acetochlor, calculated over the entire duration of the study. The plasma sulfoxide concentrations in rats fed on the acetochlor diet increased 2- to 3-fold over the first 13 weeks of the study and remained relatively constant for the remainder of the study. Consequently, the concentration of acetochlor sulfoxide in the diet had to be increased after 2 weeks (to 150 ppm) and then again after 4 weeks (to 300 ppm) in order to try to maintain comparable plasma concentrations. Even with these increases, the concentration of sulfoxide in the plasma of acetochlor sulfoxide–dosed animals was significantly lower than that in acetochlor-treated rats for a large part of the study (about 0.5 and 0.7 µg/mL plasma in acetochlor sulfoxide– and acetochlor-treated rats, respectively).

Groups of rats were killed after 1, 2, 4, 7, 13 and 39 weeks of feeding. Blood was collected by cardiac puncture (n = 8 per group) at each time point. Nasal cell replication rates were measured in rats killed after 1, 2, 4 and 13 weeks. Each animal was given a single intraperitoneal injection of ^{3}H-labelled thymidine (74 kBq/g bw) in water on each of the 3 days prior to sacrifice. The final injection was given 2 hours before the animals were killed by exsanguination under terminal anaesthesia with halothane. The remaining animals were killed after either 26 weeks (n = 32) or 52 weeks (n = 31).

No abnormalities were seen in any of the haematoxylin and eosin–stained sections from the animals examined at 1, 2 and 13 weeks. At 26 weeks, multifocal polypoid adenomas were seen in the olfactory epithelium, with an incidence of 65.5% in the acetochlor group and 21.9% in the acetochlor sulfoxide group. Hyperplasia of the nasal epithelium was seen in both treatment groups at the same sites as the adenomas. At 52 weeks, the incidence of polypoid adenomas was 65.4% and 29.6% in the acetochlor- and acetochlor sulfoxide–treated groups, respectively. The incidence of hyperplasia was 88% and 41% in acetochlor- and acetochlor sulfoxide–treated rats, respectively (Table 62).

Table 62. Histopathological findings in nasal cavities of rats treated with acetochlor and sec-amide methyl sulfoxide

Week	Finding	Control 0 ppm	Acetochlor sulfoxide[a] 300 ppm	Acetochlor[a] 1 750 ppm
26	*No. of tissues examined*	32	32	32
	Polypoid adenoma	0	7** (21.9%)	21** (65.5%)
	Hyperplasia, total	0	2 (6.25%)	6* (18.8%)
52	*No. of tissues examined*	31	27	26
	Rhinitis, total	14	12 (44.5%)	10 (38.5%)
	Polypoid adenoma	0	8** (29.6%)	17** (65.4%)
	Hyperplasia, total	0	11** (40.7%)	23** (88.5%)

ppm: parts per million
[a] Per cent incidence values shown in parentheses.
Source: Mainwaring (2004)

There were no increases in cell proliferation in nasal tissue levels 1–5 after 1 week of treatment. However, in nasal tissue level 6, the mean labelling index in the acetochlor group was 134% of that seen in controls. No increase in cell proliferation was seen in the animals treated with acetochlor sulfoxide.

The sulfoxide metabolite of acetochlor has been shown to be a nasal carcinogen in the rat. The development, morphology and location of the tumours were identical to those seen with acetochlor. Its tumorigenic potency is also comparable to that of acetochlor. These findings conclusively demonstrate that acetochlor sulfoxide, which is the major circulating metabolite in the rat, can be directly associated with the mechanism of tumour formation (Mainwaring, 2004).

A study was conducted to determine the effects of subchronic dietary administration of acetochlor on rat nasal cell proliferation. Groups of 10 male Sprague-Dawley rats were administered acetochlor (purity 95.2%) in the diet at a dose level of 0, 1750 or 5000 ppm (equal to 0, 91.9 and 270 mg/kg bw per day, respectively) for 60, 90 and 160 days. Cell proliferation was determined at 60, 90 and 160 days in both the respiratory and olfactory epithelia of the nasal turbinates by measuring the incorporation of [^{3}H]thymidine into replicating DNA. [^{3}H]Thymidine was administered daily (intraperitoneally) for 3 consecutive days prior to the rats being killed on the fourth day. Additional groups of 10 male rats were administered acetochlor in the diet at 0, 200, 1750 or 5000 ppm (equal to 0, 10.4, 91.9 and 270 mg/kg bw per day, respectively) for 163 days. Three days prior to sacrifice, these rats were implanted with osmotic pumps containing 5-bromo-2′-deoxyuridine (BrdU). After sacrifice, the nasal turbinates were removed from all animals and processed for the determination of cell proliferation.

Terminal body weights of animals administered 5000 ppm for 60, 90 and 160 days were significantly decreased compared with those of control animals (11%, 17% and 19% decrease in body weights at 60, 90 and 160 days, respectively). Cell proliferation was significantly increased (~1.6-fold) in the olfactory epithelium of rats from the 5000 ppm group at 60 days when measured using [^{3}H]thymidine (Table 63). After 90 and 160 days of acetochlor treatment, a significant increase (~1.3- to 2.0-fold) in cell proliferation was observed in rats treated with 1750 and 5000 ppm (measured using either [^{3}H]thymidine or BrdU). Cell proliferation in the olfactory epithelium was significantly increased at 5000 ppm compared with 1750 ppm (measured using [^{3}H]thymidine). There were no significant differences in cell proliferation in the respiratory epithelium at any of the time points or dose levels tested.

Table 63. Intergroup comparison of nasal cell proliferation

Group	Dietary concentration (ppm)	No. of labelled cells/field	
		Olfactory epithelium	Respiratory epithelium
[^{3}H]Thymidine	0	4.23	2.80
60 days	1 750	3.81	1.37
	5 000	6.66*	2.07
[^{3}H]Thymidine	0	4.75	1.37
90 days	1 750	6.37**	1.28
	5 000	7.90**	1.14
[^{3}H]Thymidine	0	3.48	0.52
160 days	1 750	5.24**	0.79
	5 000	7.06**	0.86
BrdU	0	7.17	2.43
160 days	200	6.25	2.23
	1 750	9.78**	2.53
	5 000	11.46**	2.61

BrdU: 5-bromo-2′-deoxyuridine; ppm: parts per million; *: $P \leq 0.05$; **: $P \leq 0.01$ (Dunnett's test, two-sided)
Source: Hotz & Wilson (1996a)

In conclusion, acetochlor produced a significant increase in cell proliferation in the olfactory region, but not the respiratory region, of the nasal turbinates of the rat in a dose-dependent manner (Hotz & Wilson, 1996a).

A study was conducted to determine and characterize nasal cell proliferation in mice following dietary administration of acetochlor. In this study, acetochlor (purity 95.2%) was administered to groups of 26 male CD-1 mice in the diet at a dose level of 0, 1000 or 5000 ppm (equal to 0, 167 and 888 mg/kg bw per day, respectively). Cell proliferation was determined at 60 and 90 days in both the respiratory and olfactory epithelia of the nasal turbinates by measuring the incorporation of BrdU into replicating DNA. BrdU was administered using micro-osmotic pumps, which were implanted 3 days prior to sacrifice.

There was a significant reduction in body weight gain throughout the study in animals that received 5000 ppm acetochlor. The mean terminal body weight of the mice treated with acetochlor at 5000 ppm was significantly less than that of controls at 90 days (12% reduced compared with controls). Liver weight was significantly increased at 90 days in mice administered 5000 ppm acetochlor. Liver weight relative to body weight was significantly increased in the mice treated with

5000 ppm acetochlor at both 60 and 90 days. These results could be related to the hepatocarcinoma found at the same dose level in chronic mouse feeding studies. Liver cell proliferation was not measured. Cell proliferation was not significantly increased in either the olfactory or the respiratory epithelium of the mouse nasal turbinates at any dose level after 60 or 90 days of dietary treatment.

In conclusion, there was no effect of treatment on cell proliferation in the olfactory or respiratory epithelium of mice administered 1000 or 5000 ppm acetochlor for 60 and 90 days via dietary administration. This finding is consistent with the lack of nasal tumours in the mouse after chronic acetochlor administration (Hotz & Wilson, 1996b).

In vitro and in vivo studies

The binding of acetochlor sulfoxide (EU reference no. 46) to rat nasal proteins was investigated in one in vitro and two in vivo experiments. In the in vitro experiment, two radiolabelled forms of acetochlor sulfoxide were incubated with rat olfactory microsomal proteins, and the binding of radioactivity to protein was assessed by gel electrophoresis. In the first in vivo experiment, rats were given a single oral dose of ^{14}C-labelled acetochlor sulfoxide at 10 mg/kg bw, and the nasal tissues were collected 8 and 24 hours after dosing. The tissues were hydrolysed, and the hydrolysate was examined by HPLC. In the second in vivo experiment, the distribution of radioactivity in the nasal passages of rats dosed orally with ^{14}C-labelled acetochlor sulfoxide at 10 mg/kg bw was studied using histoautoradiography techniques.

HPLC of hydrolysates of nasal tissue taken from the olfactory region following a single dose of ^{14}C-phenyl-labelled acetochlor sulfoxide showed the presence of at least four protein adducts, one of which eluted as a doublet and was significantly greater than the rest. The structures of adducts were not identified. There was no evidence of significant adduct formation in the respiratory region. When rat olfactory microsomal proteins were incubated in vitro with the two radiolabelled forms of acetochlor sulfoxide, the pattern of protein adducts was identical for both forms, clearly indicating that adducts carry the sulfoxide side-chain.

In conclusion, there is strong evidence that acetochlor sulfoxide binds to rat nasal olfactory proteins, probably as a result of metabolism to a quinoneimine that still carries the sulfoxide side-chain. Within the nasal tissues, the principal site of covalent binding was the Bowman's glands. None of the studies provided any evidence of significant binding to proteins in the respiratory region (Green, 2001).

A series of in vitro and in vivo experiments was conducted to assess the binding and association of acetochlor metabolites with rat, mouse and human blood. These experiments included the analysis of haemoglobin adducts, assessment of acetochlor metabolism in cultured hepatocytes, binding to erythrocytes from control rats, binding to erythrocytes from rats dosed with acetochlor and binding to erythrocytes from control mice and human volunteers.

In rats given a single oral dose of [^{14}C]acetochlor at 200 mg/kg bw, most of the radioactivity associated with blood was shown to be bound to the protein moiety of haemoglobin. Acetochlor was metabolized extensively by primary cultures of rat hepatocytes, and the binding of several [^{14}C]acetochlor metabolites to erythrocytes from control rat blood was demonstrated. This binding occurred readily, both during the incubation of hepatocytes with [^{14}C]acetochlor and after incubating freshly collected control rat erythrocytes with blood-free hepatocyte culture supernatant containing acetochlor metabolites. Rat hepatocyte cultures therefore provided a source of acetochlor metabolites with binding potential and that were also stable to storage. The interaction of these [^{14}C]acetochlor metabolites with erythrocytes from control mice and human volunteers was investigated, and no binding of radioactivity was observed. This clearly demonstrated that the rat metabolites of acetochlor that bound readily to control rat erythrocytes had no affinity for mouse or human erythrocytes. Further in vitro experiments using erythrocytes from rats given repeated oral doses of unlabelled acetochlor at 500 or 1000 mg/kg bw per day for 31 days showed little additional binding of radiolabelled metabolites to these erythrocytes, indicating that the binding sites were saturable.

It was concluded that as the rat metabolites of acetochlor, which bound readily to rat erythrocytes, had no affinity for erythrocytes from mouse or human blood, the blood binding observed among these species is a rat-specific phenomenon (Macpherson & Jones, 1991).

In vitro studies

The in vitro metabolism of [^{14}C]acetochlor (phenyl labelled; purity 99%) was investigated in both cytosol and microsomal fractions prepared from male CD rat, male CD-1 mouse and male and female squirrel monkey liver and nasal tissue. Separate nasal olfactory and respiratory tissues were obtained from the rodents.

There were no significant differences in liver fractions from rats and mice for the rates of conjugation of acetochlor with GSH or for the hydrolysis of the secondary sulfide to EMA. The hydroxylation of EMA to *p*-OH-EMA did occur at a faster rate, 5-fold, in mouse hepatic microsomal fractions than in rat fractions.

In nasal tissue fractions from rats and mice, the rates for the conjugation of acetochlor with GSH and for the oxidation of EMA to *p*-OH-EMA were comparable. There was a marked difference between rats and mice in the rate of hydrolysis of the secondary sulfide to EMA, the rate in rat nasal tissues being significantly higher, 9.8- and 24.5-fold for olfactory and respiratory nasal tissue fractions, respectively.

Marked differences were seen between rats and primates for all three metabolic reactions, particularly those in nasal tissue fractions. Compared with the highest rate in the rat, which occurred in olfactory fractions, the rate in fractions prepared from total primate nasal tissue for the conjugation of acetochlor with GSH was 26-fold lower, that for the hydrolysis of the secondary sulfide, 86-fold lower, and that for the *p*-hydroxylation of EMA, 24-fold lower. Based on these comparisons, the overall flux through the metabolic pathway from acetochlor to *p*-OH-EMA is several orders of magnitude lower in primates than in rats. In mice, the flux was up to 18-fold lower than in rats. However, as GSH conjugation of acetochlor is minimal in the mouse, the potential conversion of acetochlor to *p*-OH-EMA would be even lower than the 18-fold measured in vitro.

In conclusion, the study results show that the rate of metabolism of acetochlor to a quinoneimine precursor in animals was markedly higher in the rat than in other species, particularly primates. The study author suggested that the results provide a clear explanation for the unique sensitivity of the rat to acetochlor-induced tumours (Green, 1997b).

Acetochlor sulfoxide (*sec*-amide methyl sulfoxide; EU reference no. 46), the major metabolite circulating in the plasma of rats dosed with acetochlor, was investigated in liver and nasal tissues from CD rats, CD-1 mice and a single human nasal tissue sample. Acetochlor sulfoxide was rapidly metabolized in olfactory microsomal fractions from both rats and mice to two major products, including the *para*-hydroxy metabolite of acetochlor sulfoxide, a precursor in the formation of a quinoneimine, the proposed toxic species in rat nasal tissue. The rates for the hydroxylation reaction were comparable in rat and mouse olfactory microsomes and 6-fold higher than the rate in rat respiratory microsomes. Metabolism could not be detected in mouse liver microsomes, and that in rat liver was extremely low, 260-fold lower than in olfactory microsomes. The hydroxylation of acetochlor sulfoxide could not be detected in the single human nasal tissue sample (Green, 1998).

In vitro metabolism of U-^{14}C-phenyl-labelled acetochlor sulfoxide (*sec*-amide methyl sulfoxide; EU reference no. 46) was studied in the nasal tissues of male CD (Crl:CD(SD)BR) rats, male CD-1 mice, male and female squirrel monkeys and human nasal explants.

The rates for the hydroxylation reaction were comparable in rat and mouse olfactory microsomes and 6-fold higher than the rate in rat respiratory microsomes. Acetochlor sulfoxide did not inhibit coumarin metabolism. The hydroxylation of acetochlor sulfoxide could not be detected in 33 human nasal tissue samples, even though these were fresh surgical samples. Similarly, the primate

nasal samples did not metabolize acetochlor sulfoxide. Purified human CYP2A6 did not metabolize acetochlor sulfoxide, although it did hydroxylate coumarin at significant rates.

In conclusion, the localization of acetochlor sulfoxide, the major circulating metabolite of acetochlor, in rat nasal tissues has been attributed to metabolism by an isoform of cytochrome P450 that is unique to the nasal tissues in the rat and is concentrated mainly in the olfactory region. The product formed, *p*-hydroxyacetochlor sulfoxide, is an immediate precursor of a quinoneimine, the protein-reactive metabolite believed to be responsible for the nasal tumours seen in rats given acetochlor. A complete lack of detectable metabolism of acetochlor sulfoxide by nasal tissues from primates and humans suggests that the particular form of cytochrome P450 responsible in the rat could not be detected in primates and humans. Consequently, acetochlor-induced rat nasal tumours are not indicative of a hazard to humans (Green et al., 2000).

The in vitro metabolism of acetochlor *sec*-amide methyl sulfide (abbreviated by study authors as ASMS, EU reference no. 36), the precursor of the major circulating rat metabolite acetochlor *sec*-amide methyl sulfoxide (abbreviated by study authors as ASMSO, EU reference no. 46), was investigated for its possible conversion to reactive dialkylbenzoquinoneimine (DABQI) metabolites. The purpose of the study was to determine the relative importance in Sprague-Dawley rats of the formation of DABQI metabolites through the EMA metabolic pathway compared with other pathways, specifically the ASMSO pathway. Incubations of ASMS were conducted with rat liver and rat olfactory nasal turbinate microsomes in the presence of NADPH and GSH. GSH served to trap reactive DABQI metabolites formed in the microsomal incubations producing GSH conjugates. For comparison purposes, rat liver and nasal microsomal incubations were also conducted using ASMSO as substrate.

Four major non-conjugated metabolites, ASMSO, *p*-hydroxy acetochlor *sec*-amide methyl sulfoxide (*p*-OH-ASMSO), EMA and *p*-OH-EMA, were formed and identified from both rat liver and nasal microsomal incubations of ASMS in the presence of NADPH and GSH. ASMSO, the result of the oxidation of sulfur, was the major metabolite and was formed at similar levels in liver and nasal incubations. EMA, derived by amidase hydrolysis of the amide, was also formed at comparable levels in liver and nasal incubations, but at levels at least 10 times lower than ASMSO levels. The *para*-hydroxylated metabolites *p*-OH-EMA and *p*-OH-ASMSO were formed at levels similar to those of EMA in nasal microsomes. These two metabolites were formed at much higher levels (at least 30 times higher) in nasal microsomes than in liver microsomes.

In all, 12 GSH conjugates resulting from trapping of reactive quinoneimine (DABQI) metabolites of *para*-hydroxylated ASMSO, ASMS or EMA were formed in rat nasal microsomal incubations of ASMS in the presence of NADPH and GSH. In contrast, only three detectable quinoneimine GSH conjugates were formed in rat liver microsomal incubations in the presence of NADPH and GSH, and these were at levels much lower than those in nasal olfactory incubations. The *p*-OH-EMA GSH conjugate was the major GSH adduct metabolite formed in both liver and nasal turbinate incubations. However, the *p*-OH-EMA GSH levels in nasal microsomal incubations were more than 25-fold higher than those in rat liver microsomal incubations.

Similar non-conjugated and GSH-conjugated metabolites were formed in limited incubations of ASMSO with rat liver or nasal olfactory turbinate microsomes in the presence of NADPH and GSH. At similar substrate concentrations, the incubation of ASMSO with rat nasal microsomes formed almost 5 times more total GSH conjugates than the incubation of ASMS. The vast majority of GSH conjugates (trapped quinoneimine metabolites) formed in nasal microsomal incubations of ASMSO are formed from *p*-OH-ASMSO (> 98%), unlike the incubations of ASMS, which formed reactive quinoneimine metabolites primarily via *p*-OH-EMA.

The major toxicity of acetochlor in nasal tissues is believed to result from site-specific formation of reactive DABQI metabolites (e.g. *p*-OH-EMA quinoneimine and *p*-OH-ASMSO quinoneimine) within the olfactory epithelium. These metabolites can bind to cysteine residues in nasal proteins and produce oxidative stress. The relative levels of GSH conjugate formation (a

quantitative estimate of the formation of those reactive metabolites) in nasal olfactory turbinate microsomes provide a measure of the relative contributions of the various quinoneimine metabolites to the observed toxicity. ASMSO is the predominant metabolite circulating in the blood plasma of rats treated with acetochlor. Therefore, the results of this study, which show that more than 98% of reactive quinoneimine metabolites formed from ASMSO in nasal microsomes are derived from *p*-OH-ASMSO (not *p*-OH-EMA), indicate that the *p*-OH-ASMSO pathway is the predominant pathway contributing to acetochlor-induced nasal toxicity (Zhang, Hansen & Saghir, 2010).

A study was conducted to determine and characterize the binding of radioactivity to CD-1 mouse nasal tissue protein following dietary administration of ^{14}C-labelled acetochlor. In this study, CD-1 mice were administered ^{14}C-labelled acetochlor at target dietary concentrations of 1750 and 5000 ppm for 14 days. After treatment, the animals were killed, the nasal turbinate protein was isolated and the binding of acetochlor to the nasal turbinate protein was determined.

No evidence of EMIQ-cysteine or related benzoquinoneimine protein adduct was detected in the nasal turbinates of either dose group (Lau & Wilson, 1998a).

A study was conducted to characterize the binding of radioactivity to rhesus monkey nasal tissue protein following oral administration of ^{14}C-labelled acetochlor. In this study, three male rhesus monkeys were orally administered ^{14}C-labelled acetochlor at a dose level of 126 mg/kg bw for 14 days. After treatment, the animals were killed, and the nasal turbinates were isolated.

No evidence of an EMIQ-derived or related benzoquinoneimine protein adduct was detected in the nasal turbinates of any of the monkeys (Lau & Wilson, 1998b).

The mode of action for nasal tumours observed in rats following long-term exposure to acetochlor is analysed using the International Programme on Chemical Safety (IPCS) conceptual framework for cancer risk assessment (Sonich-Mullin et al., 2001; Boobis et al., 2006) in Appendix 1.

(d) In vitro elucidation of metabolic pathway in liver

In an in vitro study, the metabolism of acetochlor by rat liver and kidney cell–free preparations was investigated to elucidate the initial metabolic pathways of acetochlor in the rat and to complement the results obtained from the in vivo rat metabolism studies. Liver and kidney homogenates were fractionated by centrifugation to separate membrane-bound and soluble enzymes, allowing the separate study of the metabolic pathways involving cytochrome P450 enzymes and glutathione transferases, respectively.

The in vitro studies led to the identification of two pathways of acetochlor metabolism in the rat. One pathway involved glutathione transferases and the enzymes of the mercapturic acid pathway, and the other pathway involved the cytochrome P450 and glucuronyl transferase enzymes. The initial detoxification metabolites of these two pathways were identified as GSH and glucuronic acid conjugates, respectively. These two types of metabolites were not observed in the urine and faeces of the rat from the in vivo studies, indicating extensive further metabolism prior to their elimination from the rat. Metabolites resulting from oxidative or reductive dehalogenation and aryl hydroxylation were not observed (Feng, 1984).

A study was conducted to compare the rat and human liver metabolism of the chloroacetamide herbicides acetochlor, alachlor, butachlor and metolachlor in vitro. Only the test conditions and results of acetochlor are presented and discussed in this summary.

Acetochlor was incubated with rat and human liver microsomes and human cytochrome P450 isoforms 1A1, 1A2, 2B6, 2D6, 2E1 and 3A4 at a substrate concentration of 50 µmol/L for up to 1 hour in the presence of an NADPH regenerating system, and the metabolites formed were analysed by HPLC at several time points. 2-Chloro-*N*-(2-methyl-6-ethylphenyl) acetamide (CMEPA; *sec*-amide chloride; EU reference no. 8), a metabolite formed after *N*-dealkylation of acetochlor, was incubated with rat and human liver microsomes (but without an NADPH regenerating system) at a substrate concentration of 50 µmol/L for up to 1 hour, and the metabolites formed were analysed by HPLC at several time points.

Both rat and human hepatocytes metabolized CMEPA to EMA. The human cytochrome P450 isoforms involved in the metabolic pathway studied (acetochlor → CMEPA → EMA → *p*-OH-EMA) were CYP3A4 and CYP2B6. The rates of metabolic conversion at the different steps were roughly comparable between rat and human liver microsomes, with only a few slight differences (Coleman et al., 2000).

(e) Mode of action of related to thyroid tumour formation

The objective of this study was to determine the effect of acetochlor on end-points relevant to thyroid homeostasis in order to evaluate the mechanism of thyroid neoplasia. In one study, male Sprague-Dawley rats (20 per group) were fed diets containing acetochlor (purity 95.2%) at 0, 1750 or 5000 ppm (equal to 0, 92 and 270 mg/kg bw per day, respectively) for 14, 28 and 56 days. The end-points measured included terminal body weights, liver weights, thyroid weights, serum concentrations of thyroid stimulating hormone (TSH), thyroxine (T_4) and triiodothyronine (T_3) and hepatic T_4 uridine diphosphate-glucuronosyltransferase (UGT) activity. In the other study, four groups of male Sprague-Dawley rats were fed diets containing acetochlor at 0, 200, 1750 or 5000 ppm (equal to 0, 10.4, 92 and 270 mg/kg bw per day, respectively) for 90 and 160 days. The end-points as listed above were measured at 90 days; terminal body weights, liver weights and thyroid weights were evaluated only at 160 days.

Significant decreases in final body weight were observed for the animals treated with 5000 ppm acetochlor when compared with their respective controls at 14, 28, 56, 90 and 160 days. The decreases in body weight at 5000 ppm were 9%, 8%, 7%, 11% and 17%, compared with respective controls, at 14, 28, 56, 90 and 160 days, respectively. Significant reductions in body weight gain were observed during the first 3 weeks of the study for animals that received 5000 ppm acetochlor. Acetochlor caused significant increases in liver weights in animals treated with 1750 ppm for 28, 56 and 90 days. Liver weights were increased at 14, 26, 56 and 90 days in animals treated with 5000 ppm acetochlor. Thyroid weights were increased in animals treated with 1750 ppm acetochlor for 28 days. Thyroid weights were increased at 14 and 28 days in animals treated with 5000 ppm acetochlor. Concentrations of serum TSH were significantly increased at 14 and 28 days in the animals treated with 5000 ppm acetochlor. TSH was significantly increased at 56 days only in animals receiving 1750 ppm.

Serum T_4 concentrations were significantly increased only at 14 days in animals administered 1750 and 5000 ppm acetochlor. Serum T_3 concentrations were significantly decreased at 14 days in animals administered 5000 ppm acetochlor. Dose-related increases in hepatic T_4 UGT activity were observed at 14, 28 and 56 days in both the 1750 and 5000 ppm acetochlor groups and at 90 days only in the 5000 ppm group. Increased UGT was also generally observed when expressed on either a milligram protein or gram liver basis (Table 64).

The results of these studies indicate that the effect of acetochlor on circulating levels of TSH is an important event in thyroid tumour formation. It appears that increased hepatic enzymatic conjugation causes a compensatory increase in TSH levels. Taken together, the results support the view that acetochlor indirectly produces thyroid hyperplasia and ultimately neoplasia in the male rat by alterations in thyroid hormone homeostasis. This process is considered to be threshold sensitive and not relevant to humans (Hotz & Wilson, 1996c).

Table 64. Intergroup comparison of liver and thyroid gland parameters

Exposure time (days)	Dietary level (ppm)	Mean TSH level (ng/mL)	Mean T_3 level (ng/dL)	Mean T_4 level (ng/dL)	Mean hepatic T_4 UGT activity (pmol/min)			Relative liver weight (%)	Relative thyroid weight (%)
					per mg protein	per g liver	per total liver		
14	0	4.18	60.81	2.98	0.49	5.81	117.4	4.2	0.004 2
	1 750	4.42	53.69	4.18**	0.57	7.81	167.2*	4.6**	0.004 8*
	5 000	5.41**	52.3*	4.22**	0.78**	10.81**	256.1**	5.4**	0.005 4**
28	0	2.50	56.33	3.16	0.46	5.04	118.4	3.9	0.004 3
	1 750	3.32	52.04	3.30	0.63**	6.65*	149.9**	4.5**	0.005 1**
	5 000	4.56**	53.21	3.11	0.82**	9.55**	237.1**	5.1**	0.005 3**
56	0	3.22	55.94	2.13	0.51	6.68	125.7	3.7	0.004 1
	1 750	4.52*	57.27	2.60	0.69*	8.58	226.0**	4.2**	0.004 3
	5 000	4.35	60.36	2.57	0.78**	8.98	237.2**	5.0**	0.004 9**
90	0	1.45	60.99	3.60	0.38	7.59	189.0	3.8	0.003 9
	200	1.03	63.36	3.58	0.46	8.56	181.2	3.7	0.004 3
	1 750	1.70	72.14	3.85	0.45	7.77	210.6	4.2**	0.004 3
	5 000	1.42	62.46	3.44	0.63**	13.72**	361.1**	4.9**	0.005 0**
160	0	–	–	–	–	–	–	3.4	0.004 4
	200	–	–	–	–	–	–	3.5	0.004 4
	1 750	–	–	–	–	–	–	3.9**	0.005 2
	5 000	–	–	–	–	–	–	4.4**	0.005 2

ppm: parts per million; T_3: triiodothyronine; T_4: thyroxine; TSH: thyroid stimulating hormone; UGT: uridine diphosphate-glucuronosyltransferase; –: no values; *: $P \leq 0.05$; **: $P \leq 0.01$ (Dunnett's test, two-sided)
Source: Hotz & Wilson (1996c)

2.7 Studies on metabolites

Acetochlor is extensively metabolized in plants. Although several metabolites were found to represent more than 10% of the total residue in forage or fodder, none of these metabolites was found at significant levels in grain. Some plant metabolites were not detected in rat metabolism studies. EFSA (2008) decided to include one of the metabolites, *N*-(6-ethyl-3-hydroxy-2-methylphenyl) oxamic acid (EU reference no. 68), in the residue definition for human dietary risk assessment (but not for enforcement). This metabolite was found at only very low levels in maize grain and has not been detected in other crops. The oxamic acid metabolite was not formed in the rat metabolism study. Therefore, although human exposure is negligible, several studies were conducted with the oxamic acid metabolite to evaluate its toxicological potential.

Acetochlor is rapidly degraded by soil microbes, with a mean half-life of approximately 12–13 days under aerobic conditions. The four most prominent environmental degradates or soil metabolites are acetochlor *t*-ethanesulfonic acid (*t*-ESA; EU reference no. 7), acetochlor *t*-oxanilic acid (*t*-OXA; EU reference no. 2) and acetochlor *t*-sulfinylacetic acid (*t*-SAA; EU reference no. 3), each accounting for more than 10% of the applied product, and acetochlor *s*-ethanesulfonic acid (*s*-ESA; EU reference no. 13), which was found at levels above 5% in one soil type. All four of these acid metabolites are much more polar than acetochlor and have the potential to leach to groundwater. However, none of these degradates has been identified as a metabolite of acetochlor in rats or mice.

Therefore, several studies have been conducted with each of these degradates to evaluate their potential toxicity.

Another environmental metabolite, *t*-norchloroacetochlor (*t*-NCA; EU reference no. 6) has also been detected in some studies, particularly under anaerobic conditions. However, *t*-NCA represents less than 5% of the total soil residue under the aerobic conditions typical of acetochlor use, is not polar and has no tendency to accumulate. A few toxicology studies were also conducted with *t*-hydroxyacetochlor (EU reference no. 17), which was also detected at low levels (< 5%) in some soil degradation studies, but is considered a transient metabolite and unlikely to persist.

The studies conducted on selected metabolites include absorption, distribution, metabolism and excretion (ADME) studies, acute oral toxicity studies, 28- and 90-day dietary studies in rats and mice, developmental toxicity studies and genotoxicity studies. Some of the study types and key findings are summarized in Tables 65–69.

Table 65. ADME studies on selected metabolites of acetochlor

Metabolite	Study	Key findings	Reference
t-ESA (EU reference no. 7)	ADME (mouse)	Relatively poorly absorbed, 24–33% (urine) and 60–70% (faeces) in 7 days, and rapidly excreted after oral administration, with minimal metabolism (majority excreted as unchanged parent).	Hansen et al. (2009b)
t-ESA (EU reference no. 7)	ADME (rat)	Poorly absorbed, 10–13% (urine) and 77–80% (faeces) in 5 days, and rapidly excreted after oral administration, with minimal metabolism (76–79% unchanged parent).	Albin & Kraus (2000a)
t-OXA (EU reference no. 2)	ADME (mouse)	Incompletely absorbed, 54–61% (urine) and 34–39% (faeces) in 7 days, and rapidly excreted after oral administration, with minimal metabolism (predominantly unchanged parent in excreta).	Hansen et al. (2009a)
t-OXA (EU reference no. 2)	ADME (rat)	Incompletely absorbed, 34–39% (urine) and about 56% (faeces) in 7 days, and rapidly excreted after oral administration, with minimal metabolism (predominantly unchanged parent in excreta).	Albin & Kraus (2000b)
t-SAA (EU reference no. 3)	WBA (rat)	No evidence of localization. No radioactivity detected in nasal turbinates. Undetectable level of residues in organs in 5 days.	Meeker (2006a)
s-ESA (EU reference no. 13)	WBA (rat)	No evidence of localization. No radioactivity detected in nasal turbinates. Undetectable level of residues in organs in 5 days.	Meeker (2006b)

ADME: absorption, distribution, metabolism and excretion; EU: European Union; WBA: whole-body autoradiography

3. Observations in humans

No local or systemic signs of toxicity were observed in employees who handled acetochlor in laboratories or during manufacturing process development and operations. No adverse effects were reported in pesticide applicators as a result of mixing and loading and field application of acetochlor (Monsanto, 2014).

Lerro et al. (2015) examined the relationship between occupational use of acetochlor and cancer outcomes in pesticide applicators enrolled as part of the Agricultural Health Study in the USA. Phone interviews were conducted from 1999 to 2005 to obtain information on use of pesticides, including acetochlor. In total, 4026 male pesticide applicators from an available cohort of 33 484 had reported using acetochlor. Poisson regression was used to estimate relative risk (RR) and the 95%

Table 66. Acute toxicity studies on selected metabolites of acetochlor

Metabolite	Study	Strain	Purity (%)	Results	Reference
Oxamic acid (EU reference no. 68)	Acute oral (rat)	Wistar-derived	96	LD_{50}: > 2 000 mg/kg bw[a]	Duerden (1992)
t-ESA (EU reference no. 7)	Acute oral (rat)	Wistar-derived	97	LD_{50}: > 2 000 mg/kg bw[a]	Lees (1997b)
t-OXA (EU reference no. 2)	Acute oral (rat)	Wistar-derived	97	LD_{50}: > 2 000 mg/kg bw[a]	Lees (1997a)
t-SAA (EU reference no. 3)	Acute oral (rat)	Wistar-derived	99	LD_{50}: > 2 000 mg/kg bw[a]	Lees. (1997c)
s-ESA (EU reference no. 13)	Acute oral (rat)	Sprague-Dawley	86.8	LD_{50}: > 2 000 mg/kg bw[a]	Smedley (2006)
t-NCA (EU reference no. 6)	Acute oral (rat)	Sprague-Dawley	99.5	LD_{50}: 2 499 mg/kg bw (males)[b] LD_{50}: 1 002 mg/kg bw (females)[b]	Bonnette (2000)
t-Hydroxyacetochlor (EU reference no. 17)	Acute oral (rat)	Sprague-Dawley	99	LD_{50}: 1 098 mg/kg bw (female)[a]	Durando (2013)

bw: body weight; EU: European Union; LD_{50}: median lethal dose
[a] No significant clinical signs of toxicity or macroscopic findings.
[b] Decreased activity, wobbly gait, convulsions, tremors, prostration, apparent hypothermia, hunched posture.

confidence interval (CI) for cancers that occurred from the time of the interview to 2010 or 2011. As acetochlor has been registered for use in the USA only since 1994, such a time frame is unlikely to be sufficient for solid tumours to arise. Although a number of statistically significant increases in cancer incidences in applicators were calculated based on ever-use of acetochlor versus never-use of acetochlor (lung: RR = 1.74, CI = 1.07–2.84; melanoma: RR = 1.61, CI = 0.98–2.66; pancreas = 2.36, CI = 0.98–5.65), the low number of exposed applicators limits the strength of the calculated statistical associations (23, 23 and 7, respectively). No statistical associations were calculated for all cancer sites, bladder, lymphohaematopoietic, colon, colorectal, oesophagus, kidney, leukaemia, non-Hodgkin lymphoma, prostate and rectal cancer.

Further analysis by days and intensity-weighted days found statistical associations with cancer at all sites in the high-use cohort (RR = 1.19, CI = 1.00–1.43); increased colorectal cancer in the high-use cohort (RR = 1.75, CI = 1.08–2.83), but decreased colorectal cancer in the low-use cohort (RR = 0.31, CI = 0.11–0.83); increased lung cancer in the low-use but not high-use cohort (RR = 2.64, CI = 1.47–4.74); and increased melanoma in the high-use cohort (RR = 1.78, CI = 0.90–3.52). The authors also found statistical associations when acetochlor and atrazine had been used in relation to lung cancer (RR = 2.01, CI = 1.17–3.46) and melanoma (RR = 1.75, CI = 0.97–3.14).

The authors considered that the analysis was inconclusive because of minimal evidence for exposure–response relationships, the small numbers of exposed applicators and the short latency period between the use of acetochlor and the analysis of cancer outcomes (Lerro et al., 2015).

Table 67. Short-term toxicity studies on selected metabolites of acetochlor

Metabolite	Study	Doses[a]	NOAEL (mg/kg bw per day)	Key findings	Reference
Oxamic acid (EU reference no. 68)	28-day (Wistar rat)	0, 1 400, 4 000 or 14 000 ppm (equal to 0, 109, 319 and 1 142 mg/kg bw per day for males)	1 142 (HDT)	No treatment-related adverse findings at any dose level	Walraven (2009)
t-ESA (EU reference no. 7)	28-day (Sprague-Dawley rat)	0, 3 000, 6 000 or 12 000 ppm (equal to 0, 370, 767 and 1 579 mg/kg bw per day for males)	1 579 (HDT)	No treatment-related adverse effects, including on thyroid hormones, at any dose level	Lees (2000a)
	90-day (Sprague-Dawley rat)	0, 1 000, 3 000 or 12 000 ppm (equal to 0, 75.0, 225 and 919 mg/kg bw per day for males)	225	Decreased feed consumption and weight gain, slight changes in haematology and clinical chemistry in both sexes; no evidence of nasal cell proliferation	Lees (2000b)
t-OXA (EU reference no. 2)	28-day (Sprague-Dawley rat)	0, 3 000, 6 000 or 12 000 ppm (equal to 0, 373, 769 and 1 468 mg/kg bw per day for males)	769	Decreased feed consumption and weight gain; no effect on thyroid hormones	Williams (2000a)
	90-day (Sprague-Dawley rat)	0, 1 000, 3 000 or 12 000 ppm (equal to 0, 77.2, 230 and 955 mg/kg bw per day for males)	230	Decreased body weight and increased motor activity in males; no evidence of nasal cell proliferation	Williams (2000b)
t-SAA (EU reference no. 3)	28-day (Sprague-Dawley rat)	0, 1 000, 4 000 or 12 000 ppm (equal to 0, 68, 267 and 820 mg/kg bw per day for males)	820 (HDT)	No treatment-related adverse effects, including on thyroid hormones	O'Neill (2001)
	90-day (Sprague-Dawley rat)	0, 1 000, 4 000 or 14 000 ppm (equal to 0, 67, 265 and 962 mg/kg bw per day for males)	265	Decreased feed consumption and body weight	O'Neill (2002)

bw: body weight; HDT: highest dose tested; NOAEL: no-observed-adverse-effect level; ppm: parts per million

[a] Doses converted to mg/kg bw per day values for males only (female values are not shown in the table because they are usually higher numbers).

Comments

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 (Jones, 1990). No pronounced sex differences were observed following the administration of single low or high doses or repeated dosing (Hawkins, Kirkpatrick & Dean, 1989b). Radiolabel was widely distributed throughout the body (< 4% of the

Table 68. Genotoxicity studies on selected metabolites of acetochlor

Study	Test system	Purity (%)	Results	Reference
Oxamic acid (EU reference no. 68)				
Bacterial gene mutation (Ames)	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and TA1538; *Escherichia coli* WP2P and WP2P uvrA	99	Negative	Callander (1991)
In vitro chromosomal aberration	Human lymphocytes	99	Negative	Fox (1995)
In vivo unscheduled DNA synthesis	Rat hepatocytes	99	Negative	Barber & Mackay (1995)
t-ESA (EU reference no. 7)				
Bacterial gene mutation (Ames)	*S. typhimurium* TA98, TA100, TA1535 and TA1537; *E. coli* WP2 and WP2P uvrA	99	Negative	Callander (1997b)
In vitro mammalian gene mutation	Mouse lymphoma	97.6	Negative	Clay (2000b)
In vitro chromosomal aberration	Human lymphocytes	97.6	Negative	Fox (2000c)
In vivo micronucleus	Mouse (bone marrow)	97.6	Negative	Fox (2000d)
t-OXA (EU reference no. 2)				
Bacterial gene mutation (Ames)	*S. typhimurium* TA98, TA100, TA1535 and TA1537; *E. coli* WP2 and WP2P uvrA	97	Negative	Callander (1997a)
In vitro mammalian gene mutation	Mouse lymphoma	93.2	Positive (+S9) at concentrations ≥ 7.5 mmol/L	Clay (2000a)
In vitro chromosomal aberration	Human lymphocytes	93.2	Negative	Fox (2000a)
In vivo micronucleus	Mouse (bone marrow)	93.2	Negative	Fox (2000b)
t-SAA (EU reference no. 3)				
Bacterial gene mutation (Ames)	*S. typhimurium* TA98, TA100, TA1535 and TA1537; *E. coli* WP2 and WP2P uvrA	99	Negative	Callander (1997c)
In vitro mammalian gene mutation	Mouse lymphoma	100.3	Negative	Clay (2001)
In vitro	Human lymphocytes	100.3	Negative	Fox (2001)

Study	Test system	Purity (%)	Results	Reference
chromosomal aberration				
s-ESA (EU reference no. 13)				
Bacterial gene mutation (Ames)	*S. typhimurium* TA98, TA100, TA1535 and TA1537; *E. coli* WP2P uvrA	86.8	Negative	Xu (2006c)
In vitro mammalian gene mutation	CHO/HGPRT	86.8	Negative	Cifone (2006)
In vitro chromosomal aberration	Human lymphocytes	86.8	Negative	Murli (2006)
t-NCA (EU reference no. 6)				
Bacterial gene mutation (Ames)	*S. typhimurium* TA98, TA100, TA1535 and TA1537; *E. coli* WP2 and WP2P uvrA	99.5	Negative	Callander (2002)
Bacterial gene mutation (Ames)	*S. typhimurium* TA98, TA100, TA1535 and TA1537; *E. coli* WP2 and WP2P uvrA	99	Negative	Wagner (2013a)
In vitro mammalian gene mutation	Mouse lymphoma	99.5	Weakly positive (up to 2.6 times control)	Clay (2002)
In vitro chromosomal aberration	Human lymphocytes	99.5	Negative	Fox (2002a)
In vivo micronucleus	Mouse (bone marrow)	99.5	Negative	Fox (2002b)
In vivo transgenic gene mutation	Mouse	> 99	Negative	Beevers (2014)
t-Hydroxyacetochlor (EU reference no. 17)				
Bacterial gene mutation (Ames)	*S. typhimurium* TA98, TA100, TA1535 and TA1537; *E. coli* WP2P uvrA	99	Negative	Wagner (2013b)

CHO: Chinese hamster ovary; EU: European Union; HGPRT: hypoxanthine–guanine phosphoribosyltransferase; S9: 9000 × *g* supernatant fraction from rat liver homogenate

administered dose in tissues and carcass); highest concentrations were observed in blood (but not in plasma), liver, heart, lung and spleen (Hawkins, Kirkpatrick & Dean, 1989b). There was some accumulation in nasal turbinates in rats, but not in mice (Kraus & Wilson, 1996). The combined urinary and faecal excretion showed biphasic elimination, with a half-life of 5.4–10.4 hours for the α phase and 129–286 hours for the β phase (Carr et al., 1983).

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 DABQI metabolites that are believed to be responsible for the induction of nasal tumours in rats. The primary metabolic pathway in the rat

Table 69. Developmental toxicity studies on selected metabolites of acetochlor

Metabolite	Study	NOAEL (mg/kg bw per day)	Key findings	Reference
Acetochlor *t*-OXA (EU reference no. 2)	Developmental (Sprague-Dawley rat) Doses: 0, 250, 500 or 1 000 mg/kg bw per day	Maternal: 500 Developmental: 1 000 (HDT)	Mortality and clinical toxicity in the dams at 1 000 mg/kg bw per day, and no developmental toxicity at any dose level	Holson (2000)
Alachlor *t*-ESA (closely related compound)	Developmental (Sprague-Dawley rat) Doses: 0, 150, 400 or 1 000 mg/kg bw per day	Maternal: 1 000 (HDT) Developmental: 1 000 (HDT)	No test article–related maternal or developmental toxicity at any dose level	Holson (1995)

bw: body weight; EU: European Union; HDT: highest dose tested; NOAEL: no-observed-adverse-effect level

involves *O*-dealkylation and subsequent glucuronidation or GSH conjugation, enterohepatic circulation and excretion. The predominant metabolite in rat plasma following oral administration of acetochlor was a secondary amide, *sec*-amide methyl sulfoxide (or acetochlor sulfoxide). In contrast, in mice, acetochlor is metabolized primarily to a number of glucuronides, which are excreted in the urine (Green et al., 1998). In rhesus monkeys, GSH 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 *sec*-amide methyl sulfoxide, as in rats (Livingston, 1983; Kurtzweil, 2014).

Toxicological data

In rats, the acute oral LD_{50} was 1929 mg/kg bw (Branch, 1982b), the acute dermal LD_{50} was 4166 mg/kg bw (Branch, 1982a) and the acute inhalation LC_{50} was greater than 2.07 mg/L (Duerden & Lewis, 1990). Acetochlor was severely irritating to the skin of rabbits (Barlow & Ishmael, 1989) and mildly irritating to the eyes of rabbits (Branch, 1982c). It was a skin sensitizer in guinea-pigs, as determined by the Buehler test (Auletta, 1983) and Magnusson and Kligman maximization test (Botham & Ishmael, 1989). It gave a positive response for phototoxicity in an in vitro mouse fibroblast assay (Bowen, 2014).

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 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) (Ahmed, 1981a).

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) (Broadmeadow, 1985).

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 (Ahmed, 1980a).

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) (Broadmeadow, 1986a).

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, 10 or 60 mg/kg bw per day, the NOAEL was 10 mg/kg bw per day, based on decreased body weight gain, clinical signs, increased relative liver weights, increased serum ALT activity and decreased blood glucose levels observed in both sexes and reduced haemoglobin, haematocrit and erythrocyte counts observed in females at 60 mg/kg bw per day (Broadmeadow, 1986b).

In a 119-day toxicity study in dogs administered acetochlor by capsule at a dose of 0, 25, 75 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 mg/kg bw per day, decreased red blood cell counts, decreased haematocrit, increased alkaline phosphatase activity, increased ALT activity and increased relative adrenal and liver weights occurred in females (Ahmed, 1980b).

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, 12 or 40 mg/kg bw per day, the NOAEL was 12 mg/kg bw per day, based on decreased body weights and feed consumption, testicular atrophy, increased absolute liver and kidney weights and decreased relative testis weight observed at 40 mg/kg bw per day (Ahmed, 1981b).

In the second study, in which dogs were administered acetochlor by capsule at a dose of 0, 2, 10 or 50 mg/kg bw per day, the NOAEL was 2 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 mg/kg bw per day (Broadmeadow, 1989b).

The overall NOAEL for the two 1-year toxicity studies in dogs was 2 mg/kg bw per day. The overall LOAEL was 10 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 MTD (Ahmed, 1983b). 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 historical control data (Hardisty, 1997b). 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 (Hardisty, 1997c).

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, 1.1, 11 and 116 mg/kg bw per day for males and 0, 1.4, 13 and 135 mg/kg bw per day for females, respectively) for 78 weeks, the NOAEL was 10 ppm (equal to 1.1 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 mg/kg bw per day) (Amyes, 1989). 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, the 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 (Hardisty, 1997b).

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, 69 and 250 mg/kg bw per day for males and 0, 30, 93 and 343 mg/kg bw per day for females, respectively), decreased body weight in males was observed at 500 ppm (equal to 22 mg/kg bw per day), 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 mg/kg bw per day) (Ahmed, 1983a).

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 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 level in females and elevations of GGT activity and cholesterol level 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) (Naylor & Ribelin, 1986).

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) (Broadmeadow, 1989a).

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 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 methyl sulfoxide (EU reference no. 46; also known as acetochlor sulfoxide) 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 (Mainwaring, 2004).

A number of other mode of action studies (e.g. Green, 1997a,b; Lau et al., 1998a,b) are also 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.

A mechanistic study (Hotz & Wilson, 1996c) has shown that the thyroid tumours observed in rats are caused by induction of hepatic UGT. This, in turn, leads to decreased levels of thyroid

hormone and a compensatory increase in 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 GSH 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 assay in rat hepatocytes. A weakly positive response was noted in an in vivo rat hepatocyte unscheduled DNA synthesis assay, but only at a high dose level (2000 mg/kg bw, which is higher than the LD_{50} value) associated with hepatic GSH 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.

As 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, 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 166 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 18.6 mg/kg bw per day), based on focal hyperplasia and polypoid adenomata in the nasal epithelium of adult F_1 offspring at study termination and decreased body weight in F_1 females at 600 ppm (equal to 57.0 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) (Milburn, 2001).

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 (Willoughby, 1989).

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 442 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 the number of liveborn pups and 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 (Schardein, 1982).

The overall NOAEL for parental toxicity was 200 ppm (equal to 18.6 mg/kg bw per day), and the overall LOAEL was 600 ppm (equal to 57.0 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), and 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, 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 (Brooker, Stubbs & John, 1989b).

In the second study, which used oral gavage acetochlor doses of 0, 50, 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 (Rodwell, 1980).

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, 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 treatment-related 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 (Brooker, Stubbs & John, 1989a).

In the second study, which used oral gavage acetochlor doses of 0, 15, 50 and 190 mg/kg bw per day, the NOAEL for maternal toxicity was 50 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 (Adam, 1986).

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 (Kilgour, 2001a).

In a 93-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 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 mg/kg bw per day). There was no evidence for neurotoxicity or neuropathological effects up to 1750 ppm (equal to 139 mg/kg bw per day), the highest dose tested (Kilgour, 2001b).

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 (Crittenden, 2011).

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 ADME 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 and 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 (Lerro et al., 2015) 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 acceptable daily intake (ADI) of 0–0.01 mg/kg bw on the basis of a NOAEL of 1.1 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 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 acute reference dose (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 treatment-related death of two dams observed at 300 mg/kg bw per day. A safety factor of 100 was applied.

[1] *N*-(6-ethyl-3-hydroxy-2-methylphenyl) oxamic acid (EU reference no. 68), acetochlor *t*-ethanesulfonic acid (*t*-ESA) (EU reference no. 7), acetochlor *t*-oxanilic acid (*t*-OXA) (EU reference no. 2), acetochlor *t*-sulfinylacetic acid (*t*-SAA) (EU reference no. 3), acetochlor *s*-ethanesulfonic acid (*s*-ESA) (EU reference no. 13), *t*-norchloroacetochlor (*t*-NCA) (EU reference no. 6) and *t*-hydroxyacetochlor (EU reference no. 17).

Levels relevant to risk assessment of acetochlor

Species	Study	Effect	NOAEL	LOAEL
Mice	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	Toxicity	10 ppm, equal to 1.1 mg/kg bw per day	100 ppm, equal to 11 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 mg/kg bw per day
		Carcinogenicity	500 ppm, equal to 22 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 studies[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	> 2.07 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 mg/kg bw per day (dog)
Lowest relevant dermal NOAEL	100 mg/kg bw per day (highest dose tested; rat)
Lowest relevant inhalation NOAEC	No data
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Anaemia, kidney and liver (mouse and rat)
Lowest relevant oral NOAEL	1.1 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 rodent-specific thyroid tumour mode of action
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

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Appendix 1. Application of the IPCS conceptual framework for cancer risk assessment

(IPCS framework for analysing the relevance of a cancer mode of action for humans)

This framework, developed by an International Programme on Chemical Safety (IPCS) working group, provides a generic approach to the principles commonly used in evaluating a postulated mode of action (MOA) for tumour induction by a chemical (Sonich-Mullin et al., 2001; Boobis et al., 2006). Thus, the framework was used by the 2015 JMPR to provide a structured approach to the assessment of the overall weight of evidence for the postulated MOA for nasal tumours observed in rats after long-term exposure to acetochlor.

A. Nasal tumours in rats

A.1 Introduction

The incidence of nasal olfactory tumours was statistically significantly increased in all three long-term studies in rats (Ahmed, 1983; Naylor & Ribelin, 1986; Broadmeadow, 1989) at dose levels of 1000 ppm and above. Although a single nasal tumour was noted in one animal at 500 ppm in one study (Ahmed, 1983), a nasal tumour was also noted in a control animal from a different study (Naylor & Ribelin, 1986). Nasal tumours were also reported at the high dose of 5000 ppm in a carcinogenicity study; however, the dose was considered excessive based on sharply reduced body weights in females and increased mortality (Ahmed, 1983). Nasal tumours were also observed in a two-generation rat reproductive toxicity study (Milburn, 2001) at 1750 ppm in parental F_0 males and females and at 600 and 1750 ppm in parental F_1 males and females. Olfactory epithelial hyperplasia was also observed in both sexes at 1750 ppm (and in F_1 females at 600 ppm).

A.2 Postulated mode of action (theory of the case)

The postulated non-genotoxic MOA for the induction of nasal tumours by acetochlor in rats involves the biotransformation of acetochlor into reactive metabolites that result in cytotoxicity and subsequent cellular proliferation in nasal tissues. The primary metabolic pathways in the rat involve *O*-dealkylation/glucuronidation, glutathione (GSH) conjugation and excretion into the bile. The predominant metabolite in rat plasma following administration of acetochlor is a *sec*-amide methyl sulfoxide (EU reference no. 46; also known as acetochlor sulfoxide or sulfoxide metabolite), which is subsequently *para*-hydroxylated by the olfactory tissue to the reactive dialkylbenzoquinoneimine (DABQI). This sulfoxide metabolite has been shown to be similar to acetochlor in its ability to induce rat nasal tumours after repeated oral administration (Mainwaring, 2004). The reactive DABQI forms protein adducts in the olfactory epithelium, disrupting cell function and leading to cytotoxicity and regenerative proliferation. Prolonged nasal cell proliferation leads to the eventual development of nasal olfactory tumours (Ashby et al., 1996; Green et al., 2000; USEPA, 2004).

A.3 Key events

Analysis of the available toxicological data for acetochlor, including extensive mechanistic data, suggested a cytotoxicity and regenerative proliferation MOA for nasal tumours in rats that would operate through the following key events:

1. *Conjugation with GSH*. Acetochlor conjugates with GSH and is excreted in the bile.
2. *Biotransformation of GSH conjugate*. The biotransformation of the GSH conjugate results in a series of sulfur-containing products. The predominant metabolite in rat plasma following GSH conjugation of acetochlor is *sec*-amide methyl sulfoxide. Enterohepatic circulation of these products creates a pool of metabolites that are delivered to the nose.
3. *Formation of tissue-reactive metabolites in nasal tissue.* Metabolism by nasal enzymes within the olfactory epithelium results in formation of the reactive DABQI metabolite.
4. *DABQI binding to cellular proteins.* DABQI interacts with nasal tissue proteins to form adducts.
5. *Cytotoxicity.* Protein adducts disrupt cell structure and function in the olfactory epithelium, resulting in cytotoxicity.
6. *Regenerative nasal cell proliferation*. Cell loss due to cytotoxicity induces a regenerative cell proliferative response in nasal tissue.
7. *Increase in nasal olfactory tumours*. Sustained cytotoxicity and cell proliferation results in nasal olfactory tumours.

A.4 Concordance of dose–response relationship

All of the biological processes considered to contribute to the key events occurred with dose–response relationships and occurred at or below the tumorigenic dose. Key events occurring later in the MOA did not occur at lower doses than those key events considered to occur earlier in the MOA. The dose levels for the key events in the MOA for acetochlor-induced nasal tumours are presented in Table A1.

Table A1. Dose levels for key events in the MOA for acetochlor-induced nasal tumours in rats

Key event	Dose level
Conjugation with GSH	GSH conjugate is formed during biotransformation of labelled acetochlor in SD rats (Ashby et al., 1996)
Biotransformation of GSH conjugate	Metabolism studies show formation of sulfur-containing metabolites (Ashby et al., 1996)
Formation of tissue-reactive metabolites in nasal tissue	1750 ppm (14-day in vivo mechanistic study; and in vitro metabolism; Green et al., 2000)
DABQI binding to cellular proteins	1750 ppm (protein adducts; 14-day mechanistic study; Lau & Wilson, 1998)
Cytotoxicity	1750 ppm (metaplasia of olfactory mucosa; 24-month study in SD rats; Broadmeadow, 1989)
Regenerative nasal cell proliferation	1750 ppm ([^{3}H]thymidine and BrdU labelling; 160-day mechanistic study in rats; Hotz & Wilson, 1996)
Increase in nasal olfactory tumours	1750 ppm (24-month study in rats; Broadmeadow, 1989)

BrdU: 5-bromo-2′-deoxyuridine; DABQI: dialkylbenzoquinoneimine; GSH: glutathione; SD: Sprague-Dawley

A.5 Temporal association

The temporal relationships for the events associated with metabolism, tissue distribution and protein adduct formation appear to occur early following exposure to acetochlor. Whole-body autoradiography of acetochlor-dosed rats shows that distribution of the metabolites and binding to nasal turbinates take place after a single dose. Administration of acetochlor at and above 1750 ppm for 14 days resulted in formation of a protein adduct in the nasal tissues that was derived from a quinoneimine reactive species (Lau et al., 1998). There are no data to assess the earliest time of the proliferative response for acetochlor; however, acetochlor produced significantly increased cell proliferation in cells of the nasal olfactory epithelium after 60 days (approximately 8 weeks) of treatment. Polypoid adenomas of the olfactory epithelium were observed in F_0 and F_1 animals in a rat multigeneration reproduction study with acetochlor (Milburn, 2001). Additionally, preliminary data from a 1-year rat feeding study using the sulfoxide metabolite of acetochlor show a statistically significant increase in nasal polypoid adenomas in rats treated for 26 weeks (about 6.5 months), and an increased incidence of nasal tumours was seen in the 24-month rat carcinogenicity studies (Mainwaring, 2004). Overall, there is a logical temporal association of the key events with tumour formation.

A.6 Strength, consistency and specificity of association of tumour response with key events

The biological processes in the formation of reactive metabolites in nasal tissue, tissue binding and tumour formation for acetochlor are consistent with the MOA for nasal tumours for its analogue, alachlor (USEPA, 2004). The interaction of the reactive metabolite DABQI with cellular proteins is limited to the nasal olfactory epithelium, which is the site of tumour formation. Nasal tumours were also induced in rats treated with the sulfoxide metabolite of acetochlor, a proximate precursor of the quinoneimine, indicating that tumour response is associated with that metabolic pathway. The specificity of the response is further supported by mechanistic studies showing that the quinoneimine does not bind to nasal olfactory epithelium in the mouse and the fact that no nasal tumours were observed in the mouse carcinogenicity studies.

A.7 Biological plausibility and coherence

Dietary administration of acetochlor in rats resulted in the early key events (the hepatic biotransformation of acetochlor) that lead to the formation of reactive metabolites in the nasal olfactory epithelium, leading to protein adduct formation and cytotoxicity in the tissue. The MOA for acetochlor is biologically plausible and consistent with the MOA for other chemicals that induce a sustained cytotoxic response leading to regenerative proliferation and tumour formation. The data for acetochlor are reflective of a non-genotoxic, threshold MOA (USEPA, 2004).

A.8 Other modes of action

A number of MOA studies (e.g. Green, 1997a,b; Lau & Wilson, 1998; Lau et al., 1998) 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.

A.8.1 Mutagenicity

Genetic toxicity, including DNA reactivity, is one possible MOA to consider for the induction of nasal tumours in rats. Acetochlor has been evaluated for its genotoxic potential in a number of in vitro and in vivo studies. The gene mutation tests provide no clear evidence of mutagenicity in either bacterial or mammalian cell test systems. Acetochlor exhibited weak positive responses in some in vitro gene mutation assays conducted with less than pure material. Positive findings for chromosomal aberrations were reported in cultured human lymphocytes and an in vitro mouse lymphoma assay

(Howard, 1989; Fox, 1998). It is possible that the increased mutant colony count observed in the mouse lymphoma assay is the result of a clastogenic event, as that assay also detects chromosome breakage. Acetochlor was clastogenic at cytotoxic concentrations in human lymphocytes; this response is attributable to the metabolism of acetochlor to the chloromethyl group and low GSH levels. However, clastogenicity was confined only to in vitro mammalian systems. Acetochlor was negative for clastogenicity in in vivo studies, including three bone marrow (somatic cell) assays in mice or rats (Farrow & Cortina, 1983; Cavagnaro & Cortina, 1985; Randall, 1989), and was negative in four dominant lethal (germ cell) mutation studies in mice or rats (Naylor, 1987; Hodge, 1991; Milburn, 1996a,b). Additionally, when a single-cell gel electrophoresis assay (comet assay) was performed on nasal olfactory cells isolated from rats exposed to 1750 ppm (tumorigenic dose) in diet for 1 or 18 weeks, no evidence of DNA damage was detected in the target tissue (Ashby et al., 1996). No evidence of DNA damage was noted in an in vitro unscheduled DNA synthesis assay in rat hepatocytes. A weakly positive response was noted in an in vivo rat hepatocyte unscheduled DNA synthesis assay, but only at a high dose level (2000 mg/kg bw, which is higher than the median lethal dose [LD_{50} value]) associated with hepatic GSH depletion and severe hepatotoxicity. Based on a weight of evidence of the available genotoxicity data, acetochlor was unlikely to be genotoxic in vivo, and the findings do not support a mutagenic MOA for nasal tumours in rats.

A.9 Uncertainties, inconsistencies and data gaps

Although there are no data to directly demonstrate cytotoxicity in nasal tissues, evidence of respiratory metaplasia seen in the 24-month rat studies at the tumorigenic dose was used as a surrogate measure of cytotoxicity, as the presence of metaplasia is indicative of the death (and loss) of olfactory epithelial cells.

A.10 Assessment of postulated mode of action

The data provide adequate support for a non-genotoxic, threshold-based MOA for the development of nasal tumours in rats following chronic exposure to acetochlor. The key events for the MOA have been identified and are supported by data that show strong dose–response relationships and temporal concordance. Additionally, the data supporting the MOA for acetochlor are consistent with the data for its analogue, alachlor, which has similar toxicological properties and induces nasal tumours through a cytotoxicity and regenerative proliferation MOA.

Human relevance analysis

1. Is the weight of evidence sufficient to establish a mode of action in animals?

The toxicological data support a non-genotoxic, threshold-based MOA for the development of nasal cell tumours in rats, and the weight of evidence supports a cytotoxicity and regenerative proliferation MOA.

2. Can human relevance of the MOA be reasonably excluded on the basis of fundamental, qualitative differences in key events between experimental animals and humans?

The non-genotoxic MOA for the induction of nasal tumours by acetochlor in rats is plausible in humans and cannot be excluded. 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 MOA is unlikely to lead to nasal tumours in humans at levels of exposure arising from pesticide residues in food.

3. Can human relevance of the MOA be reasonably excluded on the basis of fundamental, quantitative differences in key events between experimental animals and humans?

Human relevance of the MOA can reasonably be excluded in humans based on the quantitative differences in toxicokinetics and toxicodynamics. The large species differences in the overall metabolism of acetochlor indicate that the amount of DABQI produced in rats is significantly

higher than in mice, which do not develop nasal tumours, and at least several orders of magnitude higher than in primates and humans.

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