TRINEXAPAC-ETHYL

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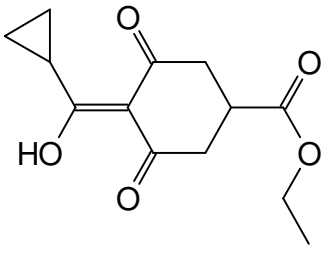
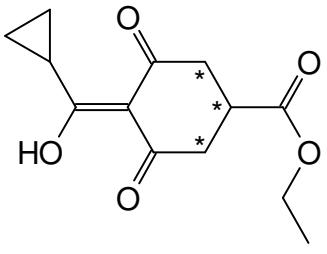
Explanation

Trinexapac-ethyl is the International Organization for Standardization–approved common name for 4-(cyclopropyl-α-hydroxymethylene)-3,5-dioxo-cyclohexanecarboxylic acid ethyl ester (International Union of Pure and Applied Chemistry), with the Chemical Abstracts Service number 95266-40-3. Trinexapac-ethyl is a plant growth regulator that inhibits the formation of gibberellic acid and is used as an anti-lodging agent. The chemical structure of trinexapac-ethyl, including the position of the radiolabel in ^{14}C-labelled trinexapac-ethyl used in rat metabolism studies, is given in Fig. 1.

Fig. 1. Chemical structure of trinexapac-ethyl and [^{14}C]trinexapac-ethyl



Trinexapac-ethyl



[3,5-cyclohexadione-1,2,6-^{14}C]Trinexapac-ethyl

* Denotes the position of the ^{14}C radiolabel

Trinexapac-ethyl 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. Minor deviations from these protocols were not considered to affect the integrity of the studies.

Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution, metabolism and excretion

Rats

[^{14}C]Trinexapac-ethyl (radiochemical purity > 98%) in ethanol/polyethylene glycol/water (30 : 40 : 30) was administered to CD albino rats (five of each sex per dose) as a single intravenous dose of 0.91 mg/kg body weight (bw), a single gavage dose of 0.97 mg/kg bw, a single gavage dose of 166 mg/kg bw or 14 daily gavage doses of unlabelled trinexapac-ethyl (purity 96.6%) at 0.97 mg/kg bw followed by a single gavage dose of radiolabelled trinexapac-ethyl at 0.91 mg/kg bw. A concurrent control group of one male and one female rat was included with each treatment group. Urine and faeces were collected at various intervals to 168 hours after dosing. Rats were killed at 168 hours for the analysis of tissue radioactivity. Radioactivity was quantified in excreta and tissues by liquid scintillation counting (LSC). Metabolites in excreta were analysed by high-performance liquid chromatography (HPLC) and thin-layer chromatography (TLC). The identity of any metabolites was confirmed by mass spectroscopy.

No signs of toxicity were reported.

The results of the excretion/mass balance phase of the study are summarized in Table 1. Mean recovery of radioactivity across the treatment groups was approximately 98% of the administered dose (range for individual animal results: 81–99%). The majority of radioactivity was detected in urine across all dosing regimens, with means ranging from 90% to 97% of the administered dose. Relatively low levels of radioactivity were detected in faeces (means of 0.6–2.0% and 0.9–2.4% of the administered dose over 0–24 and 0–168 hours, respectively). Based on the level of radioactivity in urine, the cage wash and carcass/tissues, the level of gastrointestinal absorption is at least 96% of the administered dose. The comparable levels of radioactivity in urine following oral and intravenous dosing corroborate this high level of gastrointestinal absorption. Following oral dosing, renal excretion of radioactivity was relatively rapid, with the majority (> 90%) excreted within 24 hours.

Radioactivity was generally below the limit of detection in most tissues (heart, spleen, testes, ovaries, uterus, muscle, brain, bone and erythrocytes), with only very low levels varyingly measured in the lungs, kidneys, fat, plasma and carcass in some treatment groups. In fat, low levels of radioactivity were consistently detected across all groups (0.001–0.027 parts per million [ppm]), whereas low levels were detectable in the kidneys of the single low-dose intravenous group (0.001 ppm) and single high-dose oral group (0.016 ppm in males and 0.018 ppm in females). On the basis of these findings, it is concluded that radioactivity did not accumulate in any tissues.

Across all treatment groups, the free acid derivative of trinexapac-ethyl (i.e. trinexapac acid) was identified as the sole metabolite in urine. In faeces, the parent compound was present at approximately 5–22% of the recovered faecal radioactivity (1–2.5% of the administered dose), with the balance comprising trinexapac acid (Capps, 1990).

Table 1. Mass balance in rats following oral dosing with [^{14}C]trinexapac-ethyl

Sample	Mean % of administered radioactivity							
	0.91 mg/kg bw iv		0.97 mg/kg bw po		166 mg/kg bw po		Repeat po	
	Males	Females	Males	Females	Males	Females	Males	Females
Urine								
0–24 h	87	91	91	93	91	95	93	93
0–168 h	90	94	95	95	95	97	95	96
Urine + cage wash								
0–168 h	93	97	97	96	96	98	95	97
Faeces								
0–24 h	2.0	0.8	1.4	0.9	2.0	0.8	1.1	0.6
0–168 h	1.1	1.6	1.6	1.1	2.4	1.0	1.4	0.9
Carcass + tissues								
0–168 h	0.2	0.1	< 0.3	< 0.2	0.1	< 0.1	0.1	< 0.1
Total recovery								
0–24 h	88	92	92	94	93	96	94	94
0–168 h	94	98	99	97	98	99	97	98

iv: intravenous; po: per os (by mouth)
Source: Capps (1990)

In a study by Bissig (1995), [^{14}C]trinexapac-ethyl (radiochemical purity > 96%) in ethanol/polyethylene glycol/water (30 : 40 : 30) was administered by gavage to Tif:RAl f (SPF) rats (four of each sex per dose) as a single gavage dose of 1 or 200 mg/kg bw. Blood was sampled from two rats of each sex per dose at 0.25, 0.5, 1, 2 and 4 hours, with the remaining rats sampled at 4, 8, 12, 12, 24 and 48 hours; standard kinetic parameters were determined. In a tissue distribution experiment, 12 male rats per group were administered a single gavage dose of [^{14}C]trinexapac-ethyl at 1 or 200 mg/kg bw; three rats per dose were killed at 15 minutes, 55 minutes, 2 hours and 6 hours for the analysis of tissue radioactivity. An additional experiment was conducted in four male bile duct–cannulated rats that were gavaged with a single 1 mg/kg bw dose of [^{14}C]trinexapac-ethyl. Bile was collected at 0–1, 1–2, 2–4, 4–8, 8–18, 18–24, 24–42 and 42–48 hours, with urine and faeces collected at 0–24 and 24–48 hours. The gastrointestinal tract and carcass were collected following sacrifice at 48 hours and analysed for radioactivity. Radioactivity was analysed in blood, tissues and bile by LSC. In the bile duct–cannulated rats, metabolites were analysed in bile and urine by TLC and HPLC.

No signs of toxicity were reported.

Kinetic parameters are summarized in Table 2. Radioactivity was rapidly absorbed, with maximum plasma concentrations (C_{max}) reached in 15 minutes at both doses and in both sexes. Equally, radioactivity was rapidly eliminated from plasma, with a mean half-life of 0.4–0.8 hour. The area under the plasma concentration–time curve (AUC) increased in an approximately proportional manner from the low to the high dose, suggesting linear kinetics.

The results of the tissue distribution experiment are summarized in Table 3. Confirming the preceding study by Capps (1990), low levels of radioactivity were detected in tissues. Maximum tissue concentrations were measured at 15 minutes after dosing, consistent with the plasma T_{max}. Radioactivity was rapidly eliminated from tissues; mean first-phase tissue half-lives ranged from 0.2 to 0.5 hour at the low dose and from 0.5 to 0.9 hour at the high dose, whereas the slower second-phase elimination ranged from 1.6 to 3.2 hours at the low dose and from 3.2 to 11.7 hours at the high dose.

Table 2. Kinetic parameters in rats following a single oral dose of [^{14}C]trinexapac-ethyl

Parameter	1 mg/kg bw		200 mg/kg bw	
	Males	Females	Males	Females
C_{max} (ppm)	1.3	0.5	73	85
T_{max} (min)	15	15	15	15
$t_{½}$ (h)	0.4	0.6	0.8	0.8
$AUC_{(0–48)}$ (µg eq·h/g)	1.0	0.9	170	165

AUC: area under the plasma concentration–time curve; C_{max}: maximum plasma concentration; eq: equivalent; $t_{½}$: half-life; T_{max}: time to reach C_{max}
Source: Bissig (1995)

Table 3. Tissue distribution of radioactivity in male rats following a single oral dose of [^{14}C]trinexapac-ethyl[a]

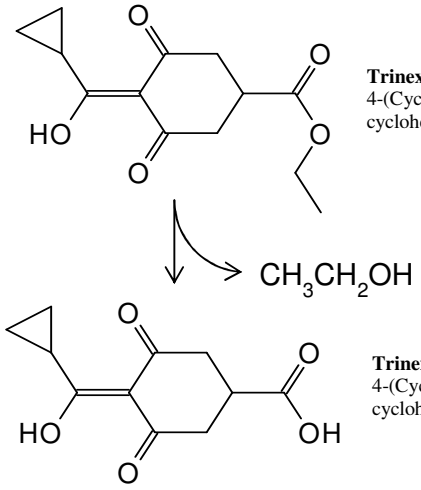
Tissue	1 mg/kg bw			200 mg/kg bw		
	Tissue concentration at 6 h (mg eq/kg bw)	$t_{½}$ (h)		Tissue concentration at 6 h (mg eq/kg bw)	$t_{½}$ (h)	
		Rapid phase	Slow phase		Rapid phase	Slow phase
Blood	0.026	0.2	1.7	7.87	0.7	4.1
Bone	0.019	0.3	3.2	3.02	0.8	11.7
Brain	0.002	0.2	1.9	0.51	0.8	4.3
Fat	0.008	0.2	1.6	1.95	0.7	4.1
Heart	0.012	0.2	1.7	2.95	0.7	3.7
Kidneys	0.265	0.2	1.9	43.37	0.6	6.3
Liver	0.144	0.2	2.3	19.5	0.5	9.3
Lungs	0.024	0.2	1.7	7.86	0.8	3.2
Muscle	0.005	0.2	1.6	1.23	0.7	6.3
Plasma	0.046	0.2	1.7	14.66	0.8	4.2
Spleen	0.006	0.3	1.7	3.53	0.9	–
Testes	0.011	0.5	2.0	1.92	0.9	5.0
Carcass	0.025	0.4	2.4	3.76	1.1	3.9

eq: equivalent; $t_{½}$: half-life
[a] Results expressed as means.
Source: Bissig (1995)

In the four bile duct–cannulated rats, total recovery of radioactivity ranged from 95% to 99% of the administered dose. The mean levels of radioactivity in urine, bile, cage wash and faeces were 79%, 3.3%, 1.1% and 0.7%, respectively, with total (0–48 hours) excretion of 84% of the administered dose. The levels of radioactivity in the gastrointestinal tract and carcass were 11% and 2.1% of the administered dose, respectively. Based on the levels of radioactivity in urine, bile, cage wash, gastrointestinal tract and carcass, gastrointestinal absorption of radioactivity is estimated to be 97%. Trinexapac acid was the main metabolite in urine (69% of the administered dose or 92% of total urinary radioactivity) and was also detected in bile (0.2% of the administered dose or 6% of total biliary radioactivity). Also detected in urine and bile was an undefined conjugate of trinexapac acid (6.3% and 2.9% of the administered dose, respectively, which accounted for 8% and 94% of total radioactivity, respectively) (Bissig, 1995).

The proposed metabolic pathway of trinexapac-ethyl in rats is shown in Fig. 2.

Fig. 2. Proposed metabolic pathway of trinexapac-ethyl in rats



Trinexapac-ethyl
4-(Cyclopropyl-α-hydroxy-methylene)-3,5-dioxo-cyclohexanecarboxylic acid ethyl ester

Trinexapac acid
4-(Cyclopropyl-α-hydroxy-methylene)-3,5-dioxo-cyclohexanecarboxylic acid

2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

The results of acute toxicity tests with trinexapac-ethyl administered to mice and rats are summarized in Table 4. Clinical signs observed in mice following acute oral dosing included piloerection, hunched posture and dyspnoea, which persisted to 5 days after dosing (Hartmann, 1993). Similar clinical signs were observed in rats following acute oral dosing, in addition to exophthalmia and ruffled fur for up to 10 days after dosing (Hartmann, 1987a); decreased activity, ataxia, diarrhoea, dilated pupils, epistaxis, haematuria, lacrimation, nasal discharge, polyuria, ptosis and salivation were also observed (Kuhn, 1988). Discoloration of the lungs and gastrointestinal tract were noted at necropsy (Kuhn, 1988).

Table 4. Results of studies of acute toxicity of trinexapac-ethyl

Species	Strain	Sex	Route	Purity (%)	Vehicle	LD_{50} (mg/kg bw) or LC_{50} (mg/L)	Reference
Mouse	Tif:MAG f	Male + female	Oral	94.5	0.5% w/v CMC in 0.1% w/v aqueous polysorbate 80	> 2 000	Hartmann (1993)
Rat	Tif:RAlf	Male + female	Oral	96.6	0.1% w/v arachidis oil	2 000–5 000	Hartmann (1987a)
Rat	HSD:(SD) BR	Male + female	Oral	96.9	None	4 613	Kuhn (1988)
Rat	Tif:RAlf	Male + female	Dermal	96.6	None	> 4 000	Hartmann (1987b)
Rat	Tif:RAlf	Male + female	Inhalation MMAD = 2.1 µm	96.6	30% w/v ethanol	> 5.3	Hartmann (1988)

CMC: carboxymethyl cellulose; MMAD: mass median aerodynamic diameter; SD: Sprague-Dawley; w/v: weight per volume

(b) Dermal and ocular irritation

The results of skin and eye irritation tests on trinexapac-ethyl conducted in rabbits are summarized in Table 5. Trinexapac-ethyl was not a skin or eye irritant in rabbits.

Table 5. Results of studies of dermal and ocular irritation of trinexapac-ethyl in rabbits

Strain	Sex	Purity (%)	Application site	Exposure period	Result	Reference
NZW	Male	96.6	20 cm^2, non-abraded skin	4 h, occluded	Non–skin irritant	Schneider (1987a)
NZW	Male	96.6	Left eye, right eye control	Eye unwashed for up to 72 h	Non–eye irritant	Schneider (1987b)

NZW: New Zealand White

(c) Dermal sensitization

Trinexapac-ethyl (purity 96.8%) was analysed for its skin sensitization potential in the guinea-pig maximization test. Twenty male guinea-pigs (Dunkin Hartley strain) were included in the test group, and 10 in the control group. In the induction phase (day 1), animals were injected intradermally on either side of the dorsal midline with 10% weight per volume (w/v) trinexapac-ethyl in arachis oil and Freund's complete adjuvant (1 : 1). Animals were pretreated with sodium lauryl sulfate in petrolatum (day 7) prior to topical induction (day 8) with undiluted trinexapac-ethyl in arachis oil for 48 hours under an occlusive dressing. Epidermal challenge (day 22) involved topical application of undiluted trinexapac-ethyl for 24 hours under an occlusive dressing. At 24 hours after topical challenge, four control and four test animals had slight erythema, with one test animal having well defined erythrema; no reactions were scored at 48 hours after challenge. On the basis of these findings, trinexapac-ethyl was classifiable as a non–skin sensitizer (Ruddock, 2001).

2.2 Short-term studies of toxicity

(a) Oral administration

Rats

Trinexapac-ethyl (purity 95%) in aqueous 1% w/v methyl cellulose was administered by gavage to groups of 10 rats of each sex per group (Tif:Ralf (SPF) hybrids of RII/1 × RII/2) at a dose of 0, 10, 100 or 1000 mg/kg bw per day for 28 days. In the absence of signs of toxicity, the highest dose was increased to 2000 mg/kg bw per day on day 10. Mortality and clinical signs were recorded daily. Body weight, feed consumption and water consumption were recorded weekly. Ophthalmoscopy was performed on all control and high-dose rats pretreatment and on day 25. Blood was sampled at the end of treatment for the analysis of haematology and clinical chemistry parameters. Following termination, rats were necropsied, and organs were weighed and examined histopathologically.

There were no deaths, treatment-related clinical signs or effects on body weight gain or feed consumption. The mean water consumption of high-dose males and females was up to about 1.5-fold higher than in the controls (week 3), with cumulative water consumption to the end of the treatment period approximately 1.2-fold higher than in the controls; no statistical analysis was performed on the water consumption data. Ophthalmoscopy was unremarkable. Prothrombin time was significantly higher ($P < 0.05$) than in the controls in high-dose rats (15.3 versus 14.6 seconds in males and 15.8 versus 15.2 seconds in females); there were no treatment-related effects on any other haematology parameters.

Selected clinical chemistry, organ weight and pathology findings are summarized in Table 6. At the highest dose, plasma potassium (both sexes) and phosphate (males) were significantly higher ($P < 0.05$) than in the controls. The significantly lower ($P < 0.05$) urea levels in low- and mid-dose males were not considered treatment related, as no effect occurred at the highest dose. In contrast,

plasma urea was 2-fold higher than in the controls ($P < 0.05$) in high-dose females. There was no analysis of serum creatinine. Alanine aminotransferase (ALAT) was increased by about 40% ($P < 0.05$) in high-dose females and by about 20% in high-dose males, with the latter not significantly different from the control.

Table 6. Findings in rats following 28 days of gavage administration of trinexapac-ethyl

Parameter	0 mg/kg bw per day		10 mg/kg bw per day		100 mg/kg bw per day		1 000/2 000 mg/kg bw per day	
	Males	Females	Males	Females	Males	Females	Males	Females
Clinical chemistry[a]								
Potassium (mmol/L)	3.60	3.33	3.70	3.48	3.66	3.50	4.15*	3.74*
Phosphate (mmol/L)	2.23	2.03	2.19	1.85	2.16	1.99	2.63*	2.11
Urea (mmol/L)	6.7	5.8	5.7*	5.9	5.3*	6.2	6.3	11.6*
ALAT (U/L)	35.8	28.3	35.1	33.9	37.4	31.6	43.0	39.0*
Organ weights[a]								
Heart								
- Absolute (g)	1.097	0.840	1.126	0.893	1.055	0.857	1.064*	0.990*
- Relative (%)	0.356	0.353	0.359	0.362	0.342	0.353	0.335*	0.396*
Kidneys								
- Absolute (g)	2.334	2.041	2.363	1.954	2.406	2.093	2.693*	2.299*
- Relative (%)	0.757	0.860	0.751	0.792	0.779	0.867	0.849*	0.921
Liver								
- Absolute (g)	11.118	8.766	11.465	11.987*	11.941	11.869*	13.191*	16.133*
- Relative (%)	3.593	3.696	3.643	4.842*	3.859	4.890*	4.150*	6.469*
Histopathology (n = 10)								
Heart: Inflammatory cell infiltration								
- Number	6	7	5	4	9	7	10	10
- Weighted grade	0.7	0.7	0.5	0.4	0.9	0.7	1.9	2.1
Liver: Hypertrophy of hepatocytes								
- Number	0	0	0	0	0	0	7	0
- Weighted grade	–	–	–	–	–	–	0.7	–
Liver: Glycogen deposition								
- Number	0	0	0	10	0	10	0	10
- Weighted grade	–	–	–	1.9	–	2.1	–	2.8
Kidneys: PAS-positive droplets								
- Number	0	0	0	0	0	0	9	0
- Weighted grade	–	–	–	–	–	–	1.1	–

ALAT: alanine aminotransferase; PAS: Periodic-Acid Schiff; U, units; *: $P < 0.05$

[a] Results expressed as the mean. Relative organ weights are relative to brain weight.

Source: Basler (1988)

At the highest dose, absolute and relative heart weights were significantly lower ($P < 0.05$) than in the controls in males, but significantly higher ($P < 0.05$) than in the controls in females. This lack of consistency suggests that these differences were incidental findings. At the highest dose, there was an increase in the incidence and grade of inflammatory cell infiltration of the myocardium. The increase in grade was due to the occurrence of moderate to marked multiple foci of inflammatory cells, infiltrating necrotic or degenerating myocardial fibres and interstitial or perivascular spaces of the myocardium in seven males and nine females. Similar inflammatory changes in the heart were not observed in any other studies at higher doses and over longer durations of exposure. On this basis, these effects were not considered treatment related.

Absolute and relative kidney weights were approximately 10% higher than the control values in high-dose males and females, with the differences in males being statistically significant ($P < 0.05$); only absolute kidney weight was significantly different ($P < 0.05$) from the control value in females. Histopathology revealed Periodic-Acid Schiff stain (PAS)–positive droplets in the kidney collecting duct epithelia in nine high-dose males, generally graded as slight. No histopathological abnormalities were observed in the kidneys of high-dose females.

At necropsy, all high-dose females were observed to have enlarged livers. Across all treated groups of females, mean absolute and relative liver weights were significantly higher ($P < 0.05$) than the control values (up to ~80% higher than the control values at the highest dose), but as the increases were coincident with glycogen deposition (the grade of which increased with dose), they were considered an adaptive effect rather than being toxicologically significant. Significantly elevated ($P < 0.05$) absolute and relative liver weights were noted in high-dose males (~19% and ~16% higher than the control values, respectively), with slight centrilobular hepatocellular hypertrophy observed microscopically in seven rats.

The no-observed-adverse-effect level (NOAEL) was 100 mg/kg bw per day for effects on the liver and kidneys at 1000 mg/kg bw per day (Basler, 1988).

Trinexapac-ethyl (96.6% purity) was admixed in the diet at a concentration of 0, 50, 500, 5000 or 20 000 ppm and fed ad libitum to SD (Crl:VAF/Plus™CD®(SD)BR) rats (15 of each sex per dose) for 13 weeks. The achieved doses were 0, 3, 34, 346 and 1350 mg/kg bw per day for males and 0, 4, 38, 395 and 1551 mg/kg bw per day for females at 0, 50, 500, 5000 and 20 000 ppm, respectively. Mortality and clinical signs were recorded daily. Body weight and feed consumption were recorded weekly. Water consumption and urine volume were recorded pretreatment and during weeks 12–13. Ophthalmoscopy was performed pretreatment and during week 13. Baseline haematology, clinical chemistry and urine analysis parameters were analysed in satellite groups of 10 rats of each sex per dose prior to the commencement of dosing; these rats were subsequently discarded. Blood and urine were sampled at the end of the exposure period for the analysis of standard haematology, clinical chemistry or urine analysis parameters. Following termination, rats were necropsied, and organs were weighed and examined histopathologically.

There were no deaths or treatment-related clinical signs. At the highest dose, absolute body weight was significantly lower ($P < 0.001$ or 0.01) than the control values in both sexes throughout the majority of the dosing period (up to ~8%), with mean cumulative body weight gain approximately 7% and 11% lower ($P < 0.05$) than the control values in males and females, respectively. Mean feed consumption was also significantly reduced ($P < 0.001$ or 0.01) in high-dose rats at various times (up to ~12% lower than the control values). There were no intergroup differences in water consumption measured during weeks 12–13. Ophthalmoscopy was unremarkable. Haematology and clinical chemistry parameters were unaffected by treatment. At the highest dose, urinary pH was significantly lower ($P < 0.01$) than the control values (6.7 versus 8.08, respectively, in males and 6.3 versus 7.8, respectively, in females). Urine specific gravity was significantly increased ($P < 0.01$) in high-dose males (1.047 versus 1.034).

There were no treatment-related macroscopic abnormalities. Selected organ weights and histopathological findings are presented in Table 7. At 5000 and 20 000 ppm, the mean relative liver

weight of males was significantly higher ($P < 0.05$) than the control values (~9% and ~12% higher, respectively), but this was not corroborated by any histopathological or clinical chemistry findings and on this basis is not considered toxicologically significant. The relative kidney weight of high-dose males and females was significantly higher ($P < 0.05$) than the control values (~10% and ~12% higher, respectively). At 5000 and 20 000 ppm in males, the incidence (but not grade) of tubular hyaline droplets was significantly higher ($P < 0.05$ or 0.01) than the control values, with the incidence of focal tubular basophilia ($P < 0.001$) and tubular casts also increased; no treatment-related histopathological kidney abnormalities were observed in females. The study authors considered that the histopathological kidney findings in males represented an early spontaneous senile nephropathy, whereas the sponsor attributed them to either renal overload by a metabolite (presumably trinexapac acid) or the binding of a metabolite to α_{2u}-globulin. However, the latter is not supported by observations in the long-term rat study, where there was no evidence of necrosis, hyperplasia, linear mineralization or renal tubule tumour formation in males. Although there might be some uncertainty about the relevance of these kidney effects in male rats to humans, the clear dose–response relationship indicates that at least in male rats, the kidney effects should be considered an adverse effect of treatment and therefore a reasonable basis for the study NOAEL.

Table 7. Observations in rats following 13 weeks of dietary exposure to trinexapac-ethyl

Parameter	0 ppm		50 ppm		500 ppm		5 000 ppm		20 000 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
Organ weights[a]										
Liver										
- Absolute (g)	13.67	8.05	14.20	8.29	14.28	7.84	14.76	8.09	14.31	8.01
- Relative (%)	2.58	2.76	2.69	2.73	2.72	2.73	2.80*	2.80	2.90**	2.94
Kidneys										
- Absolute (g)	3.82	2.14	3.81	2.22	3.74	2.10	3.93	2.18	3.94	2.26
- Relative (%)	0.73	0.74	0.73	0.73	0.71	0.73	0.75	0.76	0.80*	0.83**
Histopathology: kidney[b] **(*n* = 15)**										
Focal tubular basophilia	3	0	2	0	1	0	7	0	13***	0
Tubular hyaline droplets	5	0	7	0	7	0	11*	0	13**	0
Tubular casts	2	0	2	0	0	0	2	0	6	0

*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$

[a] Results expressed as the mean.

[b] Results expressed as the absolute number of rats with the finding.

Source: Chau, McCormick & Arthur (1989a)

The NOAEL was 500 ppm (equal to 34 mg/kg bw per day), based on histopathological findings in the kidneys (focal tubular basophilia, tubular hyaline droplets and tubular casts) in males at 5000 ppm (equal to 346 mg/kg bw per day) (Chau, McCormick & Arthur, 1989a).

Dogs

In a non-guideline study, trinexapac-ethyl (purity 96.6%) was admixed in the diet at a concentration of 0, 500, 5000, 15 000 (days 1–3), 30 000 (days 4–28) or 50 000 ppm (day 29 onwards) and fed to groups of three Beagle dogs of each sex per group for up to 49 days. The achieved doses were 0, 23, 217, 683, 734 and 965 mg/kg bw per day for both sexes at 0, 500, 5000, 15 000, 30 000 and 50 000 ppm, respectively.

There were no deaths or treatment-related clinical signs. A loss of body weight occurred from days 29 to 49 in dogs consuming the 50 000 ppm diet (1–1.3 kg in males and 1–1.4 kg in females).

Significantly reduced ($P < 0.01$) feed consumption occurred in high-dose males (~50% lower than the control values from days 1 to 29 and ~70% from days 29 to 49), with reductions in females also occurring at 50 000 ppm (~70% lower than the control values from days 29 to 49). There were no ophthalmological effects. Haematology was unremarkable. Serum cholesterol was significantly increased ($P < 0.01$ or 0.05) in both sexes at 50 000 ppm (~1.5-fold increase on day 23 in males and day 45 in both sexes). Urine analysis was unremarkable. The mean thymus weight of high-dose females (3.4 g) was significantly lower ($P < 0.01$) than the control value (14.5 g). It was stated that relative kidney weight was increased in high-dose males. At 50 000 ppm, histopathological kidney findings included albuminous casts (three males), tubular dilatation and tubular degeneration (all dogs). Minimal or diffuse thymic atrophy was observed histopathologically at 50 000 ppm (all dogs). No microscopic abnormalities of the brain were observed.

The NOAEL was 15 000 ppm (equal to 683 mg/kg bw per day), based on a range of effects that occurred at 30 000 ppm (equal to 734 mg/kg bw per day), including body weight loss, lower feed consumption, increased serum cholesterol, increased kidney weight and histopathological findings in the kidney (Spoede, Batastini & Arthur, 1991).

Trinexapac-ethyl (purity 96.9%) was admixed in the diet at a concentration of 0, 50, 1000, 15 000 or 30 000 ppm and 400 g offered on a daily basis to Beagle dogs (four of each sex per dose) for 13 weeks. High-dose dogs were offered diet containing 15 000 ppm for 3 days and thereafter received diet containing 30 000 ppm for 13 weeks. The achieved doses were 0, 2, 35, 516 and 927 mg/kg bw per day for males and 0, 2, 40, 582 and 891 mg/kg bw per day for females at 0, 50, 1000, 15 000 and 30 000 ppm, respectively. Mortality and clinical signs were recorded daily. Body weight and feed consumption were recorded weekly. Physical or auditory examinations and ophthalmoscopy were performed pretreatment and during week 13. Blood and urine were collected pretreatment and during week 13 for the analysis of standard haematology, clinical chemistry or urine analysis parameters. Following termination, dogs were necropsied, and organs were weighed and examined histopathologically.

There were no deaths. Treatment-related clinical signs occurred in two high-dose males and included emaciation (weeks 5–14 or 13–14) and decreased defecation (week 4). At the highest dose, mean feed consumption was significantly lower ($P < 0.01$ or 0.001) than the control values throughout the study (up to 57% lower than the control value in males and 65% lower in females). Consequently, high-dose males and females lost 5% and 1%, respectively, of their starting body weight during the treatment period.

Ophthalmoscopy was unremarkable. There were no treatment-related effects on haematology or urine analysis parameters. Blood glucose was significantly lower ($P < 0.05$) than the control value in high-dose males on day 86 (76.25 versus 91 mg/dL), coincident with the reduction in feed consumption; there were no other treatment-related effects on clinical chemistry parameters. Blood urea nitrogen was approximately 67% higher ($P < 0.01$) than the control value in high-dose males.

There were no treatment-related macroscopic abnormalities. Selected organ weight findings are presented in Table 8. At the highest dose, relative brain (both sexes), relative adrenal (males) and relative liver (males) weights were significantly higher ($P < 0.01$ or 0.05) than the control values; these differences are attributable to the lower mean terminal body weight. In high-dose males, the relative weight of the medial retropharyngeal lymph node was significantly higher ($P < 0.05$) than the control value, whereas the absolute and relative weights of the popliteal lymph node were significantly lower ($P < 0.01$ or 0.05) than the control values at most doses; in the absence of a dose–response relationship, these differences are considered incidental findings. Although not statistically significant, the absolute and relative thymus weights of high-dose males were ~78% and ~60% lower than in the control group. Histopathological examination revealed diffuse thymic atrophy in all high-dose dogs (graded as minimal [one male, three females], moderate [two males, one female] and severe [one male]), compared with none in the control group ($P < 0.01$). The thymic atrophy is most likely a secondary effect of the reduced body weight gain and was not observed in the 52-week dog study.

Table 8. Organ weights in dogs following 13 weeks of dietary exposure to trinexapac-ethyl

Parameter	0 ppm		50 ppm		1 000 ppm		15 000 ppm		30 000 ppm	
	M	F	M	F	M	F	M	F	M	F
Organ weights[a]										
Terminal body weight (g)	8 809	7 491	9 832	8 605	9 402	8 148	10 094	7 010	6 561*	6 668
Brain weight										
- Absolute (g)	77.17	71.16	77.33	77.53	82.58	77.32	80.22	76.27	80.16	75.38
- Relative (%)	0.88	0.95	0.79	0.91	0.89	0.96	0.80	1.09	1.24**	1.14*
Adrenal weight										
- Absolute (g)	1.005	1.140	1.140	1.055	1.077	1.062	1.265	1.030	1.170	1.047
- Relative (%)	0.011	0.015	0.012	0.012	0.012	0.013	0.013	0.015	0.018**	0.016
Liver weight										
- Absolute (g)	300.2	242.1	307.3	238.2	313.1	233	325.3	246.3	266.1	235.8
- Relative (%)	3.43	3.26	3.13	2.78	3.33	2.89	3.24	3.51	4.06**	3.56
Lymph node weight, medial retropharyngeal										
- Absolute (g)	2.500	3.137	3.295	2.900	4.145	3.142	3.845	3.297	3.320	2.615
- Relative (%)	0.028	0.042	0.034	0.034	0.044	0.039	0.038	0.047	0.049*	0.040
Lymph node weight, popliteal										
- Absolute (g)	2.322	1.527	1.607	0.995	1.225**	1.440	1.605	0.870	1.047**	0.787
- Relative (%)	0.027	0.021	0.017*	0.011	0.013**	0.018	0.016*	0.012	0.016**	0.012
Thymus weight										
- Absolute (g)	8.28	6.34	12.93	11.39*	8.05	8.69	7.09	7.58	2.71	5.71
- Relative (%)	0.093	0.086	0.130	0.132	0.086	0.107	0.070	0.106	0.038	0.084

F: females: M: males; *: $P < 0.05$; **: $P < 0.01$

[a] Results expressed as the mean. Relative weights are relative to body weight.

Source: Chau, McCormick & Arthur (1989b)

In the subsequent study by Chau, Kirchner & Arthur (1992), histopathological examination revealed vacuoles in the brain of dogs consuming a diet containing 20 000 ppm trinexapac-ethyl. A re-examination of brain slides prepared in relation to the current study (Chau, McCormick & Arthur, 1989b) by Krinke (1994) indicated that one high-dose male dog had similar vacuoles in the brain.

The NOAEL was 15 000 ppm (equal to 516 mg/kg bw per day), based on clinical signs, reduced body weight gain and reduced feed consumption at 30 000 ppm (equal to 927 mg/kg bw per day) (Chau, McCormick & Arthur, 1989b).

Trinexapac-ethyl (purity > 96.2%) was admixed in the diet at a concentration of 0, 40, 1000, 10 000 or 20 000 ppm, and 400 g was offered on a daily basis to Beagle dogs (four of each sex per dose) for 52 weeks. A maximum dietary concentration of 20 000 ppm was chosen as the highest dose because observations in the 7- and 13-week studies suggested reduced palatability at 30 000 and 50 000 ppm. The achieved doses were 0, 1.6, 32, 366 and 727 mg/kg bw per day for males and 0, 1.4, 40, 357 and 793 mg/kg bw per day for females at 0, 40, 1000, 10 000 and 20 000 ppm, respectively. Observations for mortality and clinical signs were made daily. Body weight and feed consumption were recorded pretreatment, weekly for 13 weeks and monthly thereafter. Physical or auditory

examinations were made pretreatment and during weeks 13, 26, 39 and 52. Ophthalmoscopy was performed pretreatment and during weeks 26 and 52. Blood and urine were sampled pretreatment and during weeks 13, 26 and 53 for the analysis of standard haematology, clinical chemistry or urine analysis parameters. At the end of the exposure period, dogs were killed and necropsied, and organs were weighed and examined histopathologically.

There were no deaths. Emesis was observed sporadically at 20 000 ppm (three males and four females), and mucoid or bloody faeces were seen at 10 000 ppm (three males and four females) and 20 000 ppm (three males and two females). One male each had mucoid or bloody faeces at 0, 40 and 1000 ppm.

There were no intergroup differences in the pattern of body weight gain, and pretreatment differences in absolute body weight persisted throughout the study. Feed consumption was consistent across all groups, and there were no treatment-related ophthalmic abnormalities.

Selected haematology, clinical chemistry, organ weight and pathology findings are summarized in Table 9. At the highest dose, mean red blood cells (both sexes), haematocrit (both sexes) and haemoglobin (females) were significantly lower ($P < 0.05$) than the control values at day 85. Significantly reduced red blood cell count was observed on day 357 at 10 000 ppm (females) and 20 000 ppm (both sexes). Plasma cholesterol was higher than the control value in high-dose males, but not significantly, whereas significantly elevated ($P < 0.01$ or 0.05) cholesterol occurred on day 85 (10 000 and 20 000 ppm) and day 357 in females (20 000 ppm). There were no treatment-related urine analysis findings.

There were no treatment-related macroscopic abnormalities. Mean absolute testes weight was significantly ($P < 0.05$) lower than the control values at and above 1000 ppm, but the decrease was not accompanied by any histopathological abnormalities. Similarly, mean absolute and relative uterus weights were significantly lower ($P < 0.01$ or 0.05) than the control values at and above 1000 ppm. These statistically significant differences in testes and uterine weights were considered incidental findings due to higher than normal control values. There were no intergroup differences in absolute brain weight.

Histopathological examination revealed minimal focal vacuolation of the dorsal medial hippocampus and/or lateral midbrain at 10 000 and 20 000 ppm, with the incidence at 20 000 ppm being significantly higher ($P < 0.05$) than the control value. Vacuoles were confined to a bilateral swelling of oligodendroglial and astrocytic cells and were stated to be larger in size and more closely clumped than vacuoles typically observed as artefacts in control brain sections. The vacuolation was associated with the presence of astrocytes. Cell nuclei but no axons were observed within vacuoles. There was no evidence of myelinopathy or astrocytosis/astrogliosis.

The NOAEL was 1000 ppm (equal to 32 mg/kg bw per day) for cerebral vacuolation at 10 000 ppm (equal to 357 mg/kg bw per day) in the absence of neurodegenerative or inflammatory histopathological changes or neurological signs (Chau, Kirchner & Arthur, 1992).

(b) Dermal application

Rabbits

In a 21-day dermal toxicity study, trinexapac-ethyl (purity 96.6%) in dehydrated alcohol was administered dermally, under semi-occluded conditions, to the backs of five New Zealand White rabbits at a dose of 0, 10, 100 or 1000 mg/kg bw per day. There were no deaths, clinical signs or treatment-related dermal changes. The NOAEL was 1000 mg/kg bw per day, the highest dose tested (Huber, 1989).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Trinexapac-ethyl (purity 96.2–96.9%) was admixed in the diet at a concentration of 0, 7, 70, 1000, 3500 or 7000 ppm and fed ad libitum to mice (Crl:CD-1(ICR)Br) (70 of each sex per dose) for

Table 9. Observations in dogs following 52 weeks of dietary exposure to trinexapac-ethyl

Parameter	0 ppm		40 ppm		1 000 ppm		10 000 ppm		20 000 ppm	
	M	F	M	F	M	F	M	F	M	F
Haematology[a]										
RBCs ($\times 10^6$)										
- Day 85	6.46	7.2	6.12	6.69	6.66	6.82	6.06	6.05	5.40*	5.91*
- Day 357	7.63	7.97	7.36	7.55	7.62	7.24	7.07	6.56**	6.66*	6.82**
Haematocrit (%)										
- Day 85	42.75	49.50	41.25	47.75	44.25	47.00	41.50	43.50	38.25*	41.50*
Hb (g/dL)										
- Day 85	14.50	17.1	13.83	16.23	14.92	15.83	14.17	15.18	13.08	14.43*
Clinical chemistry[a]										
Cholesterol (mg/dL)										
- Day −18	190	165	171	200	191	170	161	160	203	186
- Day 85	178	144	137	172	159	158	157	178**	220	199*
- Day 176	194	158	153	192	168	164	169	237	254	244
- Day 357	195	185	149	210	170	186	180	202	266	258*
Body/organ weights[a]										
Terminal body weight (g)	10.5	8.52	9.25	9.51	10.38	8.72	9.27	8.84	9.2	7.51
Testes weight										
- Absolute (g)	17.41	–	14.65	–	13.84*	–	12.8*	–	13.07*	–
- Relative (%)	0.17	–	0.15	–	0.14	–	0.14	–	0.14	–
Uterus weight										
- Absolute (g)	–	11.49	–	7.04	–	3.55*	–	2.87**	–	3.26*
- Relative (%)	–	0.13	–	0.07	–	0.04**	–	0.03**	–	0.04*
Brain weight										
- Absolute (g)	83.25	74.31	82.68	78.95	79.68	80.91	81.31	79.58	78.49	73.3
Histopathology										
Focal vacuolation of the brain[b]	0/4	0/4	0/4	0/4	0/4	0/4	1/4	2/4	4/4*	4/4*

*: $P < 0.05$; **: $P < 0.01$

[a] Results expressed as the mean. Relative weights are relative to body weight.

[b] Number of dogs with the finding/total number of dogs per group.

Source: Chau, Kirchner & Arthur (1992)

78 weeks. The achieved doses were 0, 0.9, 9, 131, 451 and 912 mg/kg bw per day for males and 0, 1.1, 11, 154, 539 and 1073 mg/kg bw per day for females at 0, 7, 70, 1000, 3500 and 7000 ppm, respectively. Deaths were recorded twice daily, and clinical signs once daily. Ophthalmoscopy was performed prior to the commencement of dosing and at weeks 77–78. Physical and auditory examinations were performed prior to the commencement of dosing and at weeks 13, 26, 39, 52, 65 and 78. Body weight and feed consumption were recorded weekly for 13 weeks and every 4 weeks

thereafter. Rats were palpated every 4 weeks for the first 9 months and every 2 weeks thereafter. Blood smears were prepared at weeks 52–53 and 79–81 for a differential cell count and an examination of red cell morphology. At the end of the exposure period, surviving rats were killed and necropsied. Organs were weighed and examined histopathologically.

Survival was unaffected by treatment; overall survival was 54%, 61%, 50%, 56%, 71% and 60% for males and 51%, 64%, 70%, 61%, 63% and 57% for females at 0, 7, 70, 1000, 3500 and 7000 ppm, respectively. There were no treatment-related clinical signs or ophthalmic abnormalities. Mean absolute body weight and body weight gain were significantly lower ($P < 0.01$) than in the controls in high-dose females on day 7 (body weight: 22.79 g versus 23.94 g, respectively; body weight gain: 3.62 g versus 7.44 g, respectively), but there was no concomitant reduction in feed consumption. The transient nature of these effects indicates that they are an unsuitable basis on which to establish a chronic NOAEL. There was no treatment-related effect on body weight gain or feed consumption in males. The incidence of palpable masses, haematological findings, organ weights, macroscopic findings, and neoplastic and non-neoplastic lesions were unaffected by treatment.

The NOAEL for chronic toxicity and carcinogenicity was 7000 ppm (equal to 912 mg/kg bw per day), the highest dietary concentration tested (Rudzki, Batastini & Arthur, 1991).

Rats

Trinexapac-ethyl (purity > 92%) was admixed in the diet at a concentration of 0, 10, 100, 3000, 10 000 or 20 000 ppm and fed ad libitum to SD (Crl:VAF/Plus CD(SD)Br) rats (80 or 90 of each sex per dose) for up to 104 weeks. The achieved doses were 0, 0.4, 4, 116, 393 and 806 mg/kg bw per day for males and 0, 0.5, 5, 147, 494 and 1054 mg/kg bw per day for females at 0, 10, 100, 3000, 10 000 and 20 000 ppm, respectively. After 1 year of treatment, 10 rats of each sex per dose were killed, with an additional 10 rats of each sex from the control and high-dose groups subjected to a 4-week recovery period prior to sacrifice. All remaining rats remained on treatment for a 2nd year. Mortalities and clinical signs were recorded daily. Ophthalmoscopy was performed on all rats prior to the commencement of dosing and during week 51, with 10 rats of each sex from the control and high-dose groups examined during weeks 102–103. Physical and auditory examinations were performed pretreatment and during weeks 13, 26, 39, 51, 56/57 (recovery groups), 65, 77/78, 91 and 103. Body weight and feed consumption were recorded weekly to week 13 and every 4 weeks thereafter. Rats were palpated every 4 weeks for the first 9 months and every 2 weeks thereafter. Blood smears were prepared from animals killed in a moribund condition and all surviving rats prior to terminal sacrifice. Blood and urine were collected during weeks 26 or 27, 51 or 52, 56 (recovery groups), 78 and 104 for the analysis of haematology, clinical chemistry or urine analysis parameters. Urine volume and water intake were recorded for 10 rats of each sex per dose during weeks 25, 50 or 51, 55 (recovery groups), 79 and 101. At the end of the exposure period, surviving rats were killed and necropsied. Organs were weighed and examined histopathologically. Survival was unaffected by treatment; overall survival was 40%, 27%, 29%, 27%, 37% and 53% for males and 26%, 43%, 34%, 39%, 43% and 32% for females at 0, 10, 100, 3000, 10 000 and 20 000 ppm, respectively. There were no treatment-related clinical signs. At the highest dose, mean absolute body weight, body weight gain and feed consumption were significantly lower ($P < 0.01$ or 0.05) than the control values, most consistently during the first 6–12 months of treatment. The magnitude of the reduced body weight gain was up to about 7% lower than the control value in males and about 23% lower in females. Whereas male body weight gain remained depressed for the duration of the study, females regained weight to be comparable to the controls by the end of the study.

There were no treatment-related effects on water consumption, the occurrence of physical/auditory abnormalities, ophthalmic abnormalities or haematology parameters. In males on day 180, serum creatinine was significantly higher ($P < 0.05$) than the control value at and above 100 ppm (0.32, 0.44, 0.53, 0.63, 0.52 and 0.53 mg/dL at 0, 10, 100, 300, 10 000 and 20 000 ppm, respectively); in the absence of a dose–response relationship, a similar result in females or a similar result at other times, this was not considered treatment related. Significantly lower ($P < 0.01$) mean urinary pH occurred in both sexes at 10 000 and 20 000 ppm (Table 10), a finding that was reversible following the 4-week recovery period. There were no other treatment-related urinary findings.

Table 10. Mean urinary pH values of rats over 2 years of dietary exposure to trinexapac-ethyl

Day	Mean urinary pH value											
	0 ppm		10 ppm		100 ppm		3 000 ppm		10 000 ppm		20 000 ppm	
	M	F	M	F	M	F	M	F	M	F	M	F
183	8.30	7.80	7.95	7.95	8.25	7.88	8.25	7.55	7.45**	6.70**	6.45**	6.30**
355	7.87	7.65	8.00	7.65	7.90	7.45	7.90	6.80*	7.30	6.45**	6.27**	6.27**
391	7.75	7.15	–	–	–	–	–	–	–	–	7.50	7.27
544	7.60	7.95	7.60	7.15	7.65	7.40	7.05	7.20	6.77**	6.85**	6.50**	6.20**
726	6.94	7.50	7.10	6.70	7.05	6.90	7.10	7.00	6.05**	6.10**	5.90**	5.85**

F: females; M: males; *: $P < 0.05$; **: $P < 0.01$
Source: Giknis, Batastini & Arthur (1992)

There were a number of significant differences in organ weights at the highest dose that were considered to be incidental findings due to their inconsistent occurrence over time and between sexes. There were no treatment-related necropsy findings or palpable masses.

Selected neoplastic and non-neoplastic findings are summarized in Table 11. In high-dose males killed after 104 weeks, the incidence of squamous cell carcinoma of the non-glandular stomach was significantly higher ($P < 0.05$) than in the controls (2/80 versus 0/80). Although this finding was above the historical control incidence of 0%, the absence of a similar result in high-dose males killed at earlier times or in females, accompanying increases in preneoplastic lesions (e.g. hyperplasia or papillomas), similar lesions in the forestomach of mice in the preceding study and evidence of genotoxicity, the finding is considered unlikely to be treatment related. In high-dose females, the incidence of acanthosis in the non-glandular stomach was higher than the control value (13/70 versus 7/70, respectively), but was stated to be within the historical control range and therefore was considered an incidental finding.

In high-dose males, the incidence of thyroid follicular cell adenocarcinomas was significantly higher ($P < 0.05$) than the control value but was close to the historical control range of 0–5%; there was no increase in the incidence of adenomas or the combined incidence of adenomas and carcinomas. In high-dose females, the incidence of bladder papillomas was significantly higher ($P < 0.05$) than the control value (2/80 versus 0/89, respectively; historical control incidence of 0%), with no concomitant increase in the incidence of hyperplasia or carcinomas. In high-dose females, the incidence of galactocele in the mammary gland was increased relative to the control ($P < 0.01$). Also increased at the highest dose was the incidence of bile duct hyperplasia in males ($P < 0.05$). There was a significant increase ($P < 0.05$) in tension lipidosis at and above 3000 ppm in males and at the highest dose in females; in the absence of a dose–response relationship, this was not considered treatment related.

Following 52 weeks of treatment, the incidence and severity of hyaline droplets in the renal tubular epithelium of high-dose males were increased (7/10 versus 0/10 in the controls, $P < 0.05$), with this finding reversed following the 4-week recovery period. No such finding occurred in males killed after 104 weeks or in females killed at 52 or 104 weeks. Also following 52 weeks of treatment, brown pigment was observed in the tubular epithelium of the outer renal cortex of 1/10 females at 10 000 ppm and 2/10 males ($P < 0.05$) and 7/10 females ($P < 0.01$) at 20 000 ppm. Following the 4-week recovery period, this finding remained in 2/10 high-dose females.

The NOAEL for chronic toxicity was 3000 ppm (equal to 116 mg/kg bw per day) for histopathological lesions in the kidneys (hyaline droplets and pigment deposition) at 10 000 ppm (equal to 393 mg/kg bw per day). Trinexapac-ethyl was not carcinogenic under the conditions of this study; the NOAEL for carcinogenicity was therefore 20 000 ppm (equal to 806 mg/kg bw per day), the highest dietary concentration tested (Giknis, Batastini & Arthur, 1992).

Table 11. Histopathological findings in rats following 104 weeks of dietary exposure to trinexapac-ethyl

Parameter	Incidence of finding (no. of rats affected/total no. of rats)											
	0 ppm		10 ppm		100 ppm		3 000 ppm		10 000 ppm		20 000 ppm	
	M	F	M	F	M	F	M	F	M	F	M	F
Forestomach												
Squamous cell carcinoma	0/80	0/89	0/80	0/79	0/80	0/80	0/80	0/80	0/80	0/80	2/80*	0/80
Basal epithelial hyperplasia	1/70	1/70	2/70	1/69	0/70	1/70	0/70	2/70	1/70	3/70	3/70	1/70
Acanthosis	4/70	7/70	10/70	6/69	8/70	1/70	7/70	1/70	7/70	8/70	5/70	13/70*
Thyroid												
Adenomas	4/89	0/90	2/79	1/80	3/80	1/80	5/80	2/80	3/80	1/80	3/80	2/80
Carcinomas	1/89	0/90	0/79	0/80	0/80	0/80	1/80	0/80	1/80	2/80	4/80*	0/80
Total	5/89	0/90	2/79	1/80	3/80	1/80	6/80	2/80	4/80	3/80	7/80	2/80
Bladder												
Epithelial hyperplasia	1/90	2/89	4/80	2/80	0/80	0/80	4/80	1/80	0/80	1/80	2/80	0/80
Papillomas	0/90	0/89	0/80	0/80	0/80	0/80	1/80	0/80	0/80	1/80	0/80	2/80*
Transitional cell carcinomas	1/90	0/89	0/80	0/80	0/80	0/80	0/80	0/80	0/80	0/80	0/80	0/80
Total	2/90	2/89	4/80	2/80	0/80	0/80	5/80	1/80	0/80	2/80	0/80	2/80
Mammary gland												
Galactoceles	–	5/70	–	5/70	–	4/70	–	7/70	–	9/70	–	13/70**
Liver/bile duct												
Bile duct hyperplasia	16/70	11/70	11/70	9/70	13/70	17/70	13/70	8/70	18/70	13/70	35/70**	15/70
Tension lipidosis	0/70	3/70	0/70	0/70	1/70	3/70	4/70*	5/70	5/70*	5/70	2/70*	5/70*

F: females; M: males; *: $P < 0.05$; **: $P < 0.01$
Source: Giknis, Batastini & Arthur (1992)

2.4 Genotoxicity

The results of genotoxicity studies on trinexapac-ethyl are summarized in Table 12.

Table 12. Results of genotoxicity assays on trinexapac-ethyl

End-point	Test object	Concentration or dose	Purity (%)	Results	Reference
In vitro studies					
Gene mutation	*Salmonella typhimurium* TA98, TA100, TA1535 and TA1537	Cytotoxicity test: 0.08–5 000 µg/plate (±S9) Mutagenicity test: 20–5 000 µg/plate (±S9) Acetone vehicle	Not specified	Negative	Deparade (1988)
Gene mutation	*S. typhimurium* TA98, TA100, TA102, TA1535 and TA1537 *Escherichia coli* WP2 uvrA	Cytotoxicity test (TA100 and *E. coli* WP2 uvrA only): 20.6–5 000 µg/plate (±S9) Mutagenicity test: 312.5–5 000 µg/plate (±S9) DMSO vehicle	96.8	Negative	Deparade (2001)

End-point	Test object	Concentration or dose	Purity (%)	Results	Reference
Gene mutation	Mouse lymphoma cells L5178Y/TK	Cytotoxicity test: 0.94–1 930 µg/mL (±S9) Mutagenicity test: 7.54–1 930 µg/mL (±S9) DMSO vehicle	94.5	Negative	Geleick (1993)
Gene mutation	CHO V79 cells	Cytotoxicity test: 46.9–3 000 µg/mL (+S9); 23.4–1 500 µg/mL (−S9) Mutagenicity test: 70–1 400 µg/mL (±S9) DMSO vehicle	96.6	Negative[a]	Dollenmeier (1988)
Cytogenetic test	CHO CCL 61 cells	312.5, 625 or 1 250 µg/mL (±S9) DMSO vehicle	96.8	Negative	Ogorek (2001)
Cytogenetic test	Human lymphocytes	Cytotoxicity test: 0.12–1 000 µg/mL (±S9) Cytogenetic test: 62.5–1 000 µg/mL (+S9) DMSO vehicle	96.6	Negative	Strasser (1989)
Unscheduled DNA synthesis	Rat primary hepatocytes	Cytotoxicity test: 5–5 250 µg/mL Assay 1: 0.8–400 µg/mL (−S9) Assay 2: 4–500 µg/mL (−S9) Cell culture medium vehicle	96.6	Negative	Hertner (1988)
Unscheduled DNA synthesis	Human fibroblasts	Cytotoxicity test: 5.13–5 250 µg/mL Assays 1 and 2: 37.04–4 000 µg/mL (−S9) Cell culture medium vehicle	96.6	Negative	Meyer (1988)
In vivo studies					
Micronucleus	Tif:MAGF mice (5/sex/dose), bone marrow	Study 1: 0 or 3 000 mg/kg bw; killed at 16, 24 or 48 h after dosing Study 2: 0, 750, 1 500 or 3 000 mg/kg bw; killed at 48 h after dosing Arachis oil vehicle	96.6	Negative[b]	Ceresa (1989)
Micronucleus	Tif:MAGF mice (5/sex/dose), bone marrow	0, 1 000, 2 000 or 4 000 mg/kg bw; killed at 16, 24 or 48 h after dosing Arachis oil vehicle	94.5	Negative[c]	Hertner (1992)

CHO: Chinese hamster ovary; DMSO: dimethyl sulfoxide; DNA: deoxyribonucleic acid; S9: 9000 × *g* supernatant fraction of rat liver homogenate

[a] Cytotoxicity at 750–1500 µg/mL.

[b] Toxicity at 3000 mg/kg bw.

[c] Toxicity at 4000 mg/kg bw.

2.5 Reproductive and developmental toxicity

(a) Multigeneration studies

Rats

Trinexapac-ethyl (purity 96.2%) was admixed in the diet at a concentration of 0, 10, 1000, 10 000 or 20 000 ppm and fed ad libitum to two parental generations of SD rats (30 of each sex per dose) and their offspring. Estimated doses received by rats throughout premating and gestation are presented in Table 13; mean parental doses across both generations were 0, 0.57, 58.6, 570.5 and 1166 mg/kg bw per day for males and 0, 0.73, 73, 721.5 and 1427 mg/kg bw per day for females at 0, 10, 1000, 10 000 and 20 000 ppm, respectively. The premating exposure period commenced when rats were approximately 7 weeks old and lasted for 13 (F_0) or 12 weeks (F_1). Rats were mated 1 : 1 for up to 3 weeks. Litters were reduced to four pups of each sex on day 4 postpartum. Observations for mortalities and clinical signs were made daily, with body weight and feed consumption recorded weekly. The number of live and dead pups, pup body weight and clinical signs were recorded on days 0, 4, 7, 14 and 21 postpartum. Standard reproduction and litter parameters were recorded or calculated. Necropsies were performed on all parental rats and culled pups. The following tissues were examined histopathologically in parental rats: ovaries, vagina, cervix, uterus, testes, epididymides, seminal vesicles, prostate, pituitary gland and coagulating gland.

Table 13. Doses of trinexapac-ethyl received by parental rats

Phase	Dose (mg/kg bw per day)							
	10 ppm		1 000 ppm		10 000 ppm		20 000 ppm	
	Males	Females	Males	Females	Males	Females	Males	Females
F_0 premating								
- Range	0.44–0.87	0.66–0.89	44.4–85.4	66.4–90.0	445–859	668–844	895–1 577	1 370–1 485
- Mean	0.59	0.75	62.6	74.8	595	737	1 169	1 414
Gestation	–	0.64	–	64.7	–	659	–	1 377
F_1 premating								
- Range	0.44–0.93	0.57–1.01	44.7–92.1	58.2–102	453–913	580–1 026	943–2 002	1 195–2 131
- Mean	0.55	0.71	54.6	71.2	546	706	1 162	1 440
Gestation	–	0.62	–	61.8	–	650.9	–	1 319

Source: Singh, Hazelette & Yau (1991)

There were no treatment-related deaths or clinical signs.

During the premating period, significantly reduced ($P < 0.01$ or 0.05) body weight and body weight gain occurred consistently at and above 10 000 ppm in both parental generations of rats (Table 14). In the F_0 generation, cumulative body weight gain to day 91 of the premating period was significantly lower ($P < 0.01$ or 0.05) than the control values at 10 000 and 20 000 ppm (up to ~10% and ~40% lower in males and females, respectively). This reduction in body weight gain was concomitant with reduced feed consumption. A similar pattern of reduced body weight gain and feed consumption was also evident in the F_1 generation; however, the significantly lower ($P < 0.01$) body weight gain in 1000 ppm F_1 males was not considered treatment related because there was no significant difference in females at the same dose. Further, in a 13-week dietary study in rats (Chau, McCormick & Arthur, 1989a) over a comparable dosing period, no effects on body weight, body

weight gain or feed consumption occurred at a higher dietary concentration of 5000 ppm (equal to 346 mg/kg bw per day in males).

During the gestation of F_0 dams, mean absolute body weight was significantly lower ($P < 0.05$) than the control value at and above 1000 ppm (7%, 6% and 17% lower than the control value at 1000, 10 000 and 20 000 ppm, respectively). However, there was no significant difference in body weight gain or feed consumption. In F_1 dams, there were no significant intergroup differences in body weight, body weight gain or feed consumption during gestation. During lactation, high-dose dams from both parental generations gained significantly more ($P < 0.01$) body weight than the controls, in which in fact a slight loss of body weight occurred (Table 14).

Table 14. Effect of trinexapac-ethyl on body weight gain and feed consumption in F_0 and F_1 parental rats[a]

Parameter	0 ppm		10 ppm		1 000 ppm		10 000 ppm		20 000 ppm	
	M	F	M	F	M	F	M	F	M	F
F_0 body weight gain (g)										
Premating: Days 0–91	331	141	320	133	307	124	299**	120*	287**	87**
Gestation: Days 0–20	–	128	–	129	–	119	–	121	–	119
Lactation: Days 0–21	–	−12	–	−12	–	1	–	−1	–	31**
F_0 feed consumption (% of control)										
Premating: Days 0–91	100	100	96	100	94	96	93	70	91	66
Gestation: Days 0–20	–	100	–	99	–	94	–	97	–	91
F_1 body weight gain (g)										
Premating: Days 0–84	368	147	358	137	329**	129	330**	139	305**	119*
Gestation: Days 0–20	–	132	–	126	–	124	–	118	–	118
Lactation: Days 0–21	–	−6	–	−13	–	−6	–	−2	–	21**
F_1 feed consumption (% of control)										
Premating: Days 0–84	100	100	100	99	94	97	94	97	86	89
Gestation: Days 0–20	–	100	–	97	–	95	–	98	–	91

F: females; M: males; *: $P < 0.05$; **: $P < 0.01$

[a] Results expressed as the mean.

Source: Singh, Hazelette & Yau (1991)

Reproductive performance and litter parameters were unaffected by treatment in both generations.

Treatment-related effects on pups were confined to the highest dose. Significantly lower ($P < 0.05$) survival occurred during postpartum days 4–21 in pooled F_1 pups (92.4% versus 97.8% in the controls) and during postpartum days 0–4 in female F_2 pups (92.1% versus 97.6% in the controls). Mean body weight was significantly lower ($P < 0.01$) than the control values throughout gestation in both sexes, with cumulative body weight gain approximately 20% lower than in the controls in F_1 pups and about 25% lower than in the controls in F_2 pups.

There was no treatment-related effect on the incidence of gross or histopathological abnormalities in parental rats. Relative testes weight (F_1 parental males only) and relative ovary weight (F_0 and F_1 females) were significantly higher ($P < 0.01$) than the control values, which were attributable to the significantly lower ($P < 0.01$) terminal body weight of these groups, noting that absolute organ weights were unremarkable (Table 15).

Table 15. Organ weights in F_0 and F_1 parental rats[a]

Parameter	0 ppm	10 ppm	1 000 ppm	10 000 ppm	20 000 ppm
F_0					
Testes weight (g)	3.60	3.63	3.47	3.63	3.51
Terminal body weight (g)	629.83	623.87	609.73	601.30	590.53*
Relative testes weight (%)	0.57	0.59	0.58	0.61	0.60
F_1					
Testes weight (g)	3.79	3.79	3.58	3.95	3.65
Terminal body weight (g)	666.57	667.30	311.53*	631.30	549.53**
Relative testes weight (%)	0.57	0.57	0.60	0.63	0.67**
F_0					
Ovary weight (g)	0.09	0.08	0.08	0.09	0.09
Terminal body weight (g)	349.72	346.77	341.60	334.70	295.35**
Relative ovary weight (%)	0.026	0.022	0.024	0.026	0.032**
F_1					
Ovary weight (g)	0.09	0.08	0.09	0.09	0.10
Terminal body weight (g)	370.23	347.40	332.47**	339.77*	293.90**
Relative ovary weight (%)	0.025	0.024	0.027	0.026	0.033**

*: $P < 0.05$; **: $P < 0.01$

[a] Results expressed as means. Relative organ weights are relative to body weight.

Source: Singh, Hazelette & Yau (1991)

The NOAEL for reproductive toxicity was 20 000 ppm (equal to 1166 mg/kg bw per day), the highest dietary concentration tested. The NOAEL for parental toxicity was 1000 ppm (equal to 58.6 mg/kg bw per day) for reduced body weight gain and feed consumption at 10 000 ppm (equal to 570.5 mg/kg bw per day). The NOAEL for offspring toxicity was 10 000 ppm (equal to 570.5 mg/kg bw per day) for reduced survival and body weight in the F_1 and F_2 generations at 20 000 ppm (equal to 1166 mg/kg bw per day) (Singh, Hazelette & Yau, 1991).

(b) Developmental toxicity

Rats

Trinexapac-ethyl (purity 96.6%) in peanut oil was administered by gavage to groups of 24 pregnant Tif:RAI f (SPF) rats at a dose of 0, 20, 200 or 1000 mg/kg bw per day from day 6 to day 15

of gestation. Dams were observed daily throughout gestation for clinical signs of toxicity, with body weight and feed consumption recorded throughout this period. On day 20 of gestation, surviving dams were killed and fetuses examined for external, visceral and skeletal abnormalities.

There were no treatment-related mortalities, clinical signs or effects on body weight or feed consumption. There were no treatment-related effects on the course of pregnancy, uterine findings, litter parameters or fetal weight. There were no intergroup differences in the overall incidence of fetal malformations and anomalies, the incidence of external or visceral malformations and anomalies or the incidence of skeletal malformations. There was a non-significant, dose-related increase in the incidence of asymmetrically shaped sternebrae (Table 16), with the incidence within one standard deviation of the mean historical control fetal or litter incidence. On this basis, the apparent increase in asymmetrically shaped sternebrae is considered to be an incidental finding. The incidence of delayed ossification of some cervical vertebral centres was significantly higher than the control incidence, but as the differences showed no dose–response relationship and were comparable to the historical control mean, they were not considered treatment related.

Table 16. Incidence of asymmetrically shaped sternebrae in fetuses

Parameter	0 mg/kg bw per day	20 mg/kg bw per day	200 mg/kg bw per day	1 000 mg/kg bw per day	Historical control values
Fetal data					
Fetuses examined	234	248	245	239	2 093
No. affected	2	4	5	8	–
Fetal incidence (%)	0.9	1.6	2.0	3.3	2.28 ± 2.09[a]
Litter data					
Litters examined	22	24	24	24	234
No. affected	2	4	4	7	–
Litter incidence (%)	9	17	17	29.2	15.08 ± 11.57[a]

[a] Mean ± 1 standard deviation.
Source: Schoch (1988)

The NOAEL for maternal toxicity and embryo/fetal toxicity was 1000 mg/kg bw per day, the highest dose tested (Schoch, 1988).

Rabbits

Trinexapac-ethyl (purity 96.6%) in 2% w/v methyl cellulose was administered by gavage to groups of 16 or 17 pregnant New Zealand White rabbits at a dose of 0, 10, 60 or 360 mg/kg bw per day from day 7 to day 19 of gestation. Dams were observed daily throughout gestation for clinical signs of toxicity, with body weight and feed consumption recorded on days 1, 5, 7, 9, 11, 15, 20, 24 and 29 of gestation. On day 29 of gestation, surviving dams were killed and fetuses examined for external, visceral and skeletal abnormalities.

At 360 mg/kg bw per day, two dams died; one of these had convulsions prior to being found dead on day 13, whereas the other aborted its litter and was killed in a moribund condition on day 24. Necropsy of the latter dam revealed macroscopic evidence of irritation of the stomach mucosa (haemorrhagic depressions of the stomach). One dam was killed at 60 mg/kg bw per day for humane reasons due to an intubation error. There were no other deaths or treatment-related clinical signs. Body weight loss occurred across all groups from days 7 to 9, with the largest loss occurring in high-dose dams (42 g compared with a loss of 20 g in the control); this difference was not statistically significant. High-dose dams took longer to recover from the initial body weight loss, regaining weight from day 15 compared with day 11 in all other groups. Mean feed consumption tended to be lower than the control values in high-dose dams ($P < 0.05$ over days 11–14), but was actually already lower than the control value before the commencement of dosing.

At the highest dose, postimplantation losses (24.8%) were significantly higher ($P < 0.05$) than the control value (13.2%), although preimplantation losses at the highest dose (24.3%) were also significantly higher ($P < 0.05$) than the control value (14.3%). The mean number of liver fetuses per dam at the highest dose (5.7) was significantly lower ($P < 0.05$) than the control value (7.7). Mean litter weight at the highest dose (255.7 g) was lower than the control value (332.0 g), but not significantly. Given that these fetal effects occurred only in the presence of frank maternal toxicity, which arose only after 1–2 weeks, they were not considered to be an acute effect.

There was no treatment-related effect on the incidence of external, visceral or skeletal malformations, anomalies or variations.

The NOAEL for maternal toxicity was 60 mg/kg bw for deaths in several dams at 360 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was also 60 mg/kg bw per day, based on increased postimplantation losses and a reduction in the mean number of live fetuses at 360 mg/kg bw per day (Hughes, 1990).

2.6 Special studies

(a) Neurotoxicity

In an acute neurotoxicity study, groups of 10 Crl:CD(SD) rats of each sex received a single gavage dose of trinexapac-ethyl (purity 95.8%) at 0, 500, 1000 or 2000 mg/kg bw. Observations were made twice daily for deaths and clinical signs. Body weight was recorded pretreatment and on days 0, 1, 2, 7, 8, 14 and 15. Feed consumption was recorded during days 0–1, 1–2 and 7–8. A functional observational battery and motor activity assessment were performed pretreatment, at 4 hours after dosing (i.e. the approximate time to peak effect) and at days 7 and 14. On day 15, five rats of each sex per group were killed, their brain weights were recorded and a neurohistopathological examination was performed.

There were no deaths or treatment-related clinical signs. At the highest dose, the mean body weight of males did not increase from day 0 to day 1, whereas the control group gained 5 g; this difference was statistically significant ($P < 0.01$). An examination of individual animal data indicated that three high-dose male rats lost body weight within 24 hours of dosing (−3, −3 and −6 g), two did not gain any weight and the remainder gained 1, 1, 2 and 4 g. In control males, one rat lost 3 g body weight within 24 hours, with the remainder gaining from 2 to 10 g body weight. The mean body weight of high-dose males was approximately 4% lower than the control value on day 1, but not significantly so. In high-dose females, a mean of 4 g body weight was lost from day 0 to day 1, in contrast to a loss of 1 g in the control group. After this initial reduction in body weight gain, there were no intergroup differences in body weight gain. Concomitant with the initial reduction in mean body weight gain was a significant reduction ($P < 0.05$) in mean feed consumption in high-dose males (17 versus 24 g in the control). Feed consumption in high-dose females was comparable to the control values. Although there was clearly a transient, treatment-related reduction in body weight, body weight gain and feed consumption in high-dose males shortly after a single gavage dose, the small magnitude of the reductions without any other accompanying effects is unlikely to represent an adverse effect. There were no treatment-related macroscopic abnormalities, effects on brain dimensions or weight or histopathological abnormalities.

The NOAEL was 2000 mg/kg bw, the highest dose tested. There was no evidence that trinexapac-ethyl was neurotoxic (Beck, 2012a).

In a subchronic neurotoxicity study, trinexapac-ethyl (purity > 95.8%) was admixed in the diet at a concentration of 0, 3750, 7500 or 15 000 ppm and fed ad libitum to groups of 12 Crl:CD(SD) rats of each sex for approximately 13 weeks. The achieved doses were 0, 233, 463 and 948 mg/kg bw per day for males and 0, 294, 588 and 1171 mg/kg bw per day for females at 0, 3750, 7500 and 15 000 ppm, respectively. Observations were made daily for mortalities and clinical signs. Body weight and feed consumption were recorded weekly. Functional observational battery and locomotor

activity assessments were conducted prior to treatment and during weeks 3, 7 and 12. Ophthalmoscopy was performed prior to treatment and during week 11. Five rats of each sex per dose were killed during week 13; their brains were removed and the dimensions recorded. Neurohistopathological examination was performed on selected central and peripheral nervous system tissues from five rats of each sex from the control and high-dose groups. There were no deaths and no treatment-related clinical signs. The mean body weight gain of high-dose females was significantly lower ($P < 0.01$) than the control values from days 0 to 7 (~32% lower than the control values). Thereafter, the mean body weight gain of 15 000 ppm females was generally similar to that of the controls. Mean feed consumption was also significantly reduced ($P < 0.05$) in high-dose females over days 0–7 and 7–14 (by ~11%). The mean body weight of high-dose females was slightly lower than the control values during days 14–91; however, no statistical significance was achieved. The transient nature and small magnitude of these findings in females were considered not to be toxicologically significant. There were no treatment-related effects on body weight gain or feed consumption in any other group, including males. Ophthalmoscopy, the functional observational battery and locomotor activity assessment were unremarkable. Brain weight, brain dimensions and the incidence of neurohistopathological abnormalities showed no relationship with treatment.

The NOAEL was 15 000 ppm (equal to 948 mg/kg bw per day), the highest dietary concentration tested. There was no evidence that trinexapac-ethyl was neurotoxic (Beck, 2012b).

3. Observations in humans

There were no reports submitted on adverse health effects in workers involved in the manufacture or use of trinexapac-ethyl. No cases of human poisonings have been reported.

Comments

Biochemical aspects

In studies conducted in rats using [^{14}C]trinexapac-ethyl, the time to reach the maximum plasma and tissue concentration of radioactivity was 15 minutes following a single gavage dose of 1 or 200 mg/kg bw. Gastrointestinal absorption was at least 96%. The plasma elimination half-life of radioactivity was less than 1 hour. Radioactivity was rapidly eliminated from tissues; mean first-phase tissue half-lives ranged from 0.2 to 0.5 hour at 1 mg/kg bw and from 0.5 to 0.9 hour at 200 mg/kg bw, whereas the slower second-phase elimination ranged from 1.6 to 3.2 hours at 1 mg/kg bw and from 3.2 to 11.7 hours at 200 mg/kg bw. There was no evidence of accumulation of radioactivity in any tissue. Excretion of radioactivity was predominantly via the urine (≥ 90% of the administered dose), with the majority of this occurring within 24 hours of dosing. Low levels of radioactivity were detected in faeces and bile (up to approximately 2.4% and 3.3% of the administered dose, respectively). Trinexapac-ethyl undergoes limited metabolism in the rat, involving predominantly ester hydrolysis of trinexapac-ethyl to trinexapac acid. The predominant urinary metabolite was trinexapac acid (up to 100% of total urinary radioactivity), with low levels of a conjugated derivative of trinexapac acid detected only in the urine of bile duct–cannulated rats (6.3% of the administered dose). In faeces, the parent compound accounted for 5–22% of total faecal radioactivity (1–2.5% of the administered dose), with the balance comprising trinexapac acid. Bile contained mainly the conjugated derivative of trinexapac acid (2.9% of the administered dose), with low levels of the parent compound also detected (0.2% of the administered dose).

Toxicological data

The oral LD_{50} in rats was 2000 mg/kg bw. In rats, the dermal LD_{50} was greater than 4000 mg/kg bw, and the LC_{50} was greater than 5.3 mg/L. Trinexapac-ethyl was neither a skin nor an eye irritant in rabbits. In a guinea-pig maximization test, no skin sensitization occurred.

In repeated-dose toxicity studies in rats and dogs, the main target organ was the kidneys. In rats, increased kidney weight and accompanying histopathological changes (focal tubular basophilia,

tubular hyaline droplets and pigment deposition) occurred. Additional treatment-related effects in the brain were observed in dog studies.

In a 4-week gavage study in rats, which tested doses of 0, 10, 100 and 1000 mg/kg bw per day, the NOAEL was 100 mg/kg bw per day for effects on the liver and kidneys at 1000 mg/kg bw per day.

In a 13-week dietary toxicity study in rats, which tested concentrations of 0, 50, 500, 5000 and 20 000 ppm (equal to 0, 3, 34, 346 and 1350 mg/kg bw per day for males and 0, 4, 38, 395 and 1551 mg/kg bw per day for females, respectively), the NOAEL was 500 ppm (equal to 34 mg/kg bw per day) for histopathological findings in the kidney in males at 5000 ppm (equal to 346 mg/kg bw per day).

In a 7-week, non-guideline study in dogs that tested dietary concentrations of 0, 500, 5000, 15 000, 30 000 and 50 000 ppm (equal to an average of 0, 23, 217, 683, 734 and 965 mg/kg bw per day for both sexes, respectively), the NOAEL was 15 000 ppm (equal to 683 mg/kg bw per day), based on a range of effects that occurred at 30 000 ppm (equal to 734 mg/kg bw per day), including body weight loss, lower feed consumption, increased serum cholesterol, increased kidney weight and histopathological findings in the kidney.

In a 13-week study in dogs, which tested dietary concentrations of 0, 50, 1000, 15 000 and 30 000 ppm (equal to 0, 2, 35, 516 and 927 mg/kg bw per day for males and 0, 2, 40, 582 and 891 mg/kg bw per day for females, respectively), the NOAEL was 15 000 ppm (equal to 516 mg/kg bw per day) for reduced body weight gain and feed consumption at 30 000 ppm (equal to 927 mg/kg bw per day). At 30 000 ppm in males, blood glucose was reduced, whereas focal vacuolation occurred in the brain of one dog.

In a 52-week toxicity study in dogs, which tested dietary concentrations of 0, 40, 1000, 10 000 and 20 000 ppm (equal to 0, 1.6, 32, 366 and 727 mg/kg bw per day for males and 0, 1.4, 40, 357 and 793 mg/kg bw per day for females, respectively), the NOAEL was 1000 ppm (equal to 32 mg/kg bw per day) for cerebral vacuolation at 10 000 ppm (equal to 357 mg/kg bw per day) in the absence of neurodegenerative or inflammatory histopathological changes or neurological signs.

In a 78-week study in mice, which tested dietary concentrations of 0, 7, 70, 1000, 3500 and 7000 ppm (equal to 0, 0.9, 9, 131, 451 and 912 mg/kg bw per day for males and 0, 1.1, 11, 154, 539 and 1073 mg/kg bw per day for females, respectively), the NOAEL for chronic toxicity and carcinogenicity was 7000 ppm (equal to 912 mg/kg bw per day), the highest dietary concentration tested.

In a 104-week study in rats, which tested dietary concentrations of 0, 10, 100, 3000, 10 000 and 20 000 ppm (equal to 0, 0.4, 4, 116, 393 and 806 mg/kg bw per day for males and 0, 0.5, 5, 147, 494 and 1054 mg/kg bw per day for females, respectively), the NOAEL for chronic toxicity was 3000 ppm (equal to 116 mg/kg bw per day) for histopathological lesions in the kidneys at 10 000 ppm (equal to 393 mg/kg bw per day). The NOAEL for carcinogenicity was 20 000 ppm (equal to 806 mg/kg bw per day), the highest dietary concentration tested.

The Meeting concluded that trinexapac-ethyl is not carcinogenic in mice or rats.

Trinexapac-ethyl was tested in an adequate range of in vitro and in vivo genotoxicity tests. No evidence of genotoxicity was found.

The Meeting concluded that trinexapac-ethyl is unlikely to be genotoxic.

Given the lack of genotoxicity and the absence of carcinogenicity in mice and rats, the Meeting concluded that trinexapac-ethyl is unlikely to pose a carcinogenic risk to humans.

In a two-generation reproductive toxicity study in rats, which tested dietary concentrations of 0, 10, 1000, 10 000 and 20 000 ppm (equal to 0, 0.57, 58.6, 570.5 and 1166 mg/kg bw per day for males and 0, 0.73, 73, 721.5 and 1427 mg/kg bw per day for females, respectively), there was no evidence of reproductive toxicity up to 20 000 ppm (equal to 1166 mg/kg bw per day), the highest dietary concentration tested. The NOAEL for parental toxicity was 1000 ppm (equal to 58.6 mg/kg

bw per day) for reduced body weight gain and feed consumption at 10 000 ppm (equal to 570.5 mg/kg bw per day). The NOAEL for offspring toxicity was 10 000 ppm (equal to 570.5 mg/kg bw per day) for reduced survival and body weight in the F_1 and F_2 generations at 20 000 ppm (equal to 1166 mg/kg bw per day).

In a rat developmental toxicity study, which tested doses of 0, 20, 200 and 1000 mg/kg bw per day, the NOAEL for maternal and embryo/fetal toxicity was 1000 mg/kg bw per day, the highest dose tested.

In a rabbit developmental toxicity study, which tested doses of 0, 10, 60 and 360 mg/kg bw per day, the NOAEL for maternal toxicity was 60 mg/kg bw per day for deaths of several dams at 360 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was also 60 mg/kg bw per day for increased postimplantation losses and a reduction in mean number of live fetuses at 360 mg/kg bw per day.

The Meeting concluded that trinexapac-ethyl is not teratogenic in rats or rabbits.

In an acute neurotoxicity study in rats that tested doses of 0, 500, 1000 and 2000 mg/kg bw per day, the NOAEL was 2000 mg/kg bw, the highest dose tested.

In a subchronic neurotoxicity study in rats that tested dietary concentrations of 0, 3750, 7500 and 15 000 ppm (equal to 0, 233, 463 and 948 mg/kg bw per day for males and 0, 294, 588 and 1171 mg/kg bw per day for females, respectively), the NOAEL was 15 000 ppm (equal to 948 mg/kg bw per day), the highest dietary concentration tested.

Toxicological data on metabolites and/or degradates

The Meeting noted the formation of two processing degradates of trinexapac acid, CGA 113745 and CGA 313458, not detected in rat metabolism studies. Based on a structural assessment of these degradates and an estimate of the levels of chronic dietary intake, the Meeting concluded that they are unlikely to pose a dietary risk.

Human data

There were no reports submitted on adverse health effects in workers involved in the manufacture or use of trinexapac-ethyl. No cases of human poisonings have been reported.

The Meeting concluded that the database on trinexapac-ethyl was adequate to characterize the potential hazards to fetuses, infants and children.

Toxicological evaluation

The Meeting established an acceptable daily intake (ADI) of 0–0.3 mg/kg bw per day, expressed as trinexapac acid equivalents[1], based on a NOAEL of 32 mg/kg bw per day for trinexapac-ethyl (equivalent to 29 mg/kg bw per day expressed as trinexapac acid equivalents) for cerebral vacuolation in male and female dogs following 52 weeks of dietary exposure, with the application of a 100-fold safety factor. In the absence of information to the contrary, including mechanistic data, the cerebral vacuolation observed in dogs was considered relevant to humans.

The Meeting concluded that it is not necessary to establish an acute reference dose (ARfD) for trinexapac-ethyl in view of its low acute oral toxicity and the absence of developmental toxicity or any other toxicological effects that would be likely to be elicited by a single dose.

[1] To cover the possible dietary exposure to a range of salts, esters and conjugates of trinexapac, it is appropriate to express the ADI as trinexapac acid equivalents using a conversion factor of 0.9 based on differences in molecular weight between trinexapac-ethyl and trinexapac acid.

Levels relevant to risk assessment of trinexapac-ethyl

Species	Study	Effect	NOAEL	LOAEL
Mouse	Eighteen-month study of toxicity and carcinogenicity[a]	Toxicity	7 000 ppm, equal to 912 mg/kg bw per day[b]	–
		Carcinogenicity	7 000 ppm, equal to 912 mg/kg bw per day[b]	–
Rat	Thirteen-week study of toxicity[a]	Toxicity	500 ppm, equal to 34 mg/kg bw per day	5 000 ppm, equal to 346 mg/kg bw per day
	Two-year study of toxicity and carcinogenicity[a]	Toxicity	3 000 ppm, equal to 116 mg/kg bw per day	10 000, equal to 393 mg/kg bw per day
		Carcinogenicity	20 000 ppm, equal to 806 mg/kg bw per day[b]	–
	Two-generation study of reproductive toxicity[a]	Reproductive toxicity	20 000 ppm, equal to 1 166 mg/kg bw per day[b]	–
		Parental toxicity	1 000 ppm, equal to 59 mg/kg bw per day	10 000 ppm, equal to 571 mg/kg bw per day
		Offspring toxicity	10 000 ppm, equal to 571 mg/kg bw per day	20 000 ppm, equal to 1 166 mg/kg bw per day
	Developmental toxicity study[c]	Maternal toxicity	1 000 mg/kg bw per day[b]	–
		Embryo and fetal toxicity	1 000 mg/kg bw per day[b]	–
Rabbit	Developmental toxicity study[c]	Maternal toxicity	60 mg/kg bw per day	360 mg/kg bw per day
		Embryo and fetal toxicity	60 mg/kg bw per day	360 mg/kg bw per day
Dog	One-year study of toxicity[a]	Toxicity	1 000 ppm, equal to 32 mg/kg bw per day	10 000 ppm, equal to 357 mg/kg bw per day

LOAEL: lowest-observed-adverse-effect level; NOAEL: no-observed-adverse-effect level
[a] Dietary administration.
[b] Highest dose tested.
[c] Gavage administration.

Estimate of acceptable daily intake

0–0.3 mg/kg bw per day

Estimate of acute reference dose

Unnecessary

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 trinexapac-ethyl

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid and complete
Distribution	Widespread tissue distribution
Potential for accumulation	No potential for accumulation
Rate and extent of excretion	Rapid and complete
Metabolism in animals	Limited; mainly hydrolysis to trinexapac acid
Toxicologically significant compounds in animals, plants and the environment	Trinexapac-ethyl, trinexapac acid
Acute toxicity	
Rat, LD_{50}, oral	2 000 mg/kg bw
Rat, LD_{50}, dermal	> 4 000 mg/kg bw
Rat, LC_{50}, inhalation	> 5.3 mg/L
Rabbit, dermal irritation	Non-irritating
Rabbit, ocular irritation	Non-irritating
Dermal sensitization	Non-sensitizing (guinea-pig maximization test)
Short-term studies of toxicity	
Target/critical effect	Kidney and brain
Lowest relevant oral NOAEL	32 mg/kg bw per day (dog)
Lowest relevant dermal NOAEL	1 000 mg/kg bw per day, the highest dose tested (rabbit)
Lowest relevant inhalation NOAEC	No data
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Kidney
Lowest relevant NOAEL	116 mg/kg bw per day (rat)
Carcinogenicity	Not carcinogenic
Genotoxicity	
	Not genotoxic
Reproductive toxicity	
Reproduction target/critical effect	No evidence of reproductive toxicity
Lowest relevant parental NOAEL	59 mg/kg bw per day
Lowest relevant offspring NOAEL	571 mg/kg bw per day
Lowest relevant reproduction NOAEL	1 166 mg/kg bw per day, the highest dose tested
Developmental toxicity	
Developmental target/critical effect	Postimplantation losses at maternally toxic doses (rabbit)
Lowest maternal NOAEL	60 mg/kg bw per day (rabbit)
Lowest embryo/fetal NOAEL	60 mg/kg bw per day (rabbit)
Neurotoxicity	
Acute and subchronic neurotoxicity	Not neurotoxic
Medical data	
	No data

LC_{50}: median lethal concentration; LD_{50}: median lethal dose; NOAEC: no-observed-adverse-effect concentration; NOAEL: no-observed-adverse-effect level

Summary

	Value	Studies	Safety factor
ADI	0–0.3 mg/kg bw	One-year toxicity study in dogs	100
ARfD	Unnecessary	—	—

ADI: acceptable daily intake; ARfD: acute reference dose

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