CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

Substance Name: Mesotrione

EC Number: Not listed

CAS Number: 104206-82-8

Index Number: 609-064-00-X

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	Mesotrione
EC number:	Not listed
CAS number:	104206-82-8
Annex VI Index number:	609-064-00-X
Degree of purity:	≥ 93%
Impurities:	There are a number of process impurities. These have been taken into account in the assessment and are not considered to impact on the proposed classification.

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	Aquatic Acute 1 – H400: very toxic to aquatic life Aquatic Chronic 1 – H410: very toxic to aquatic life with long lasting effects
Current proposal for consideration by RAC	STOT-RE 2; H373 - May cause damage to organs (kidneys) through prolonged or repeated exposure Repr 2; H361d – Suspected of damaging the unborn child Aquatic Acute 1; H400 - Very toxic to aquatic life Acute M factor = 10 Aquatic Chronic 1; H410 - Very toxic to aquatic life with long lasting effects Chronic M factor = 10
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	STOT-RE 2; H373 – May cause damage to organs (kidneys) through prolonged or repeated exposure Repr 2; H361d – Suspected of damaging the unborn child Aquatic Acute 1; H400 - Very toxic to aquatic life Acute M factor = 10 Aquatic Chronic 1; H410 - Very toxic to aquatic life with long lasting effects Chronic M factor = 10

1.3 Proposed harmonised classification and labelling

Table 3: Proposed classification

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	-	Not applicable	Not classified	Not considered in this proposal
2.2.	Flammable gases	-	Not applicable	Not classified	Not considered in this proposal
2.3.	Flammable aerosols	-	Not applicable	Not classified	Not considered in this proposal
2.4.	Oxidising gases	-	Not applicable	Not classified	Not considered in this proposal
2.5.	Gases under pressure	-	Not applicable	Not classified	Not considered in this proposal
2.6.	Flammable liquids	-	Not applicable	Not classified	Not considered in this proposal
2.7.	Flammable solids	-	Not applicable	Not classified	Not considered in this proposal
2.8.	Self-reactive substances and mixtures	-	Not applicable	Not classified	Not considered in this proposal
2.9.	Pyrophoric liquids	-	Not applicable	Not classified	Not considered in this proposal
2.10.	Pyrophoric solids	-	Not applicable	Not classified	Not considered in this proposal
2.11.	Self-heating substances and mixtures	-	Not applicable	Not classified	Not considered in this proposal
2.12.	Substances and mixtures which in contact with water emit flammable gases	-	Not applicable	Not classified	Not considered in this proposal
2.13.	Oxidising liquids	-	Not applicable	Not classified	Not considered in this proposal
2.14.	Oxidising solids	-	Not applicable	Not classified	Not considered in this proposal
2.15.	Organic peroxides	-	Not applicable	Not classified	Not considered in this proposal
2.16.	Substance and mixtures corrosive to metals	-	Not applicable	Not classified	Not considered in this proposal
3.1.	Acute toxicity - oral	-	Not applicable	Not classified	Not considered in this proposal
	Acute toxicity - dermal	-	Not applicable	Not classified	Not considered in this proposal

	Acute toxicity - inhalation	-	Not applicable	Not classified	Not considered in this proposal
3.2.	Skin corrosion / irritation	-	Not applicable	Not classified	Not considered in this proposal
3.3.	Serious eye damage / eye irritation	-	Not applicable	Not classified	Not considered in this proposal
3.4.	Respiratory sensitisation	-	Not applicable	Not classified	Not considered in this proposal
3.4.	Skin sensitisation	-	Not applicable	Not classified	Not considered in this proposal
3.5.	Germ cell mutagenicity	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
3.6.	Carcinogenicity	Not classified	Not applicable	Not classified	Conclusive but not sufficient for classification
3.7.	Reproductive toxicity	**Repr 2; H361d – Suspected of damaging the unborn child**	Not applicable	**Not classified**	-
3.8.	Specific target organ toxicity –single exposure	-	Not applicable	Not classified	Not considered in this proposal
3.9.	Specific target organ toxicity – repeated exposure	**STOT-RE 2; H373 – May cause damage to organs (kidneys) through prolonged or repeated exposure**	Not applicable	**Not classified**	**Conclusive but not sufficient for classification**
3.10.	Aspiration hazard	-	Not applicable	Not classified	Not considered in this proposal
4.1.	Hazardous to the aquatic environment	**Aquatic Acute 1; H400 - Very toxic to aquatic life** **Aquatic Chronic 1; H410 - Very toxic to aquatic life with long lasting effects**	**Acute M factor = 10** **Chronic M factor = 10**	**Aquatic Acute 1 – H400: very toxic to aquatic life** **Aquatic Chronic 1 – H410: very toxic to aquatic life with long lasting effects**	n/a
5.1.	Hazardous to the ozone layer	-	Not applicable	Not classified	Not considered in this proposal

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling:

Pictogram(s):	GHS08, GHS09
Signal word:	Warning
Hazard statements:	H373 – May cause damage to organs (kidneys) through prolonged or repeated exposure H361d – Suspected of damaging the unborn child H410 - Very toxic to aquatic life with long lasting effects
Precautionary statements:	Not included in Annex VI

Proposed notes assigned to an entry: None

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Mesotrione is a pesticide active substance subject to renewal under Regulation (EC) 1107/2009, for which the UK is the Rapporteur Member State (RMS). The substance has an existing entry in Annex VI of CLP which includes classification for environmental hazards only. The available data on mesotrione (as presented in this report) were considered previously at the Meeting of the Commission Working Group on the Classification and Labelling of Dangerous Substances, Pesticides – Health Effects (October 2000) and it was agreed that classification for human health was not appropriate. Whilst no new data are available, concerns for classification were raised in the EFSA peer review process of the renewal and a targeted review of the classification and labelling is considered appropriate to address these.

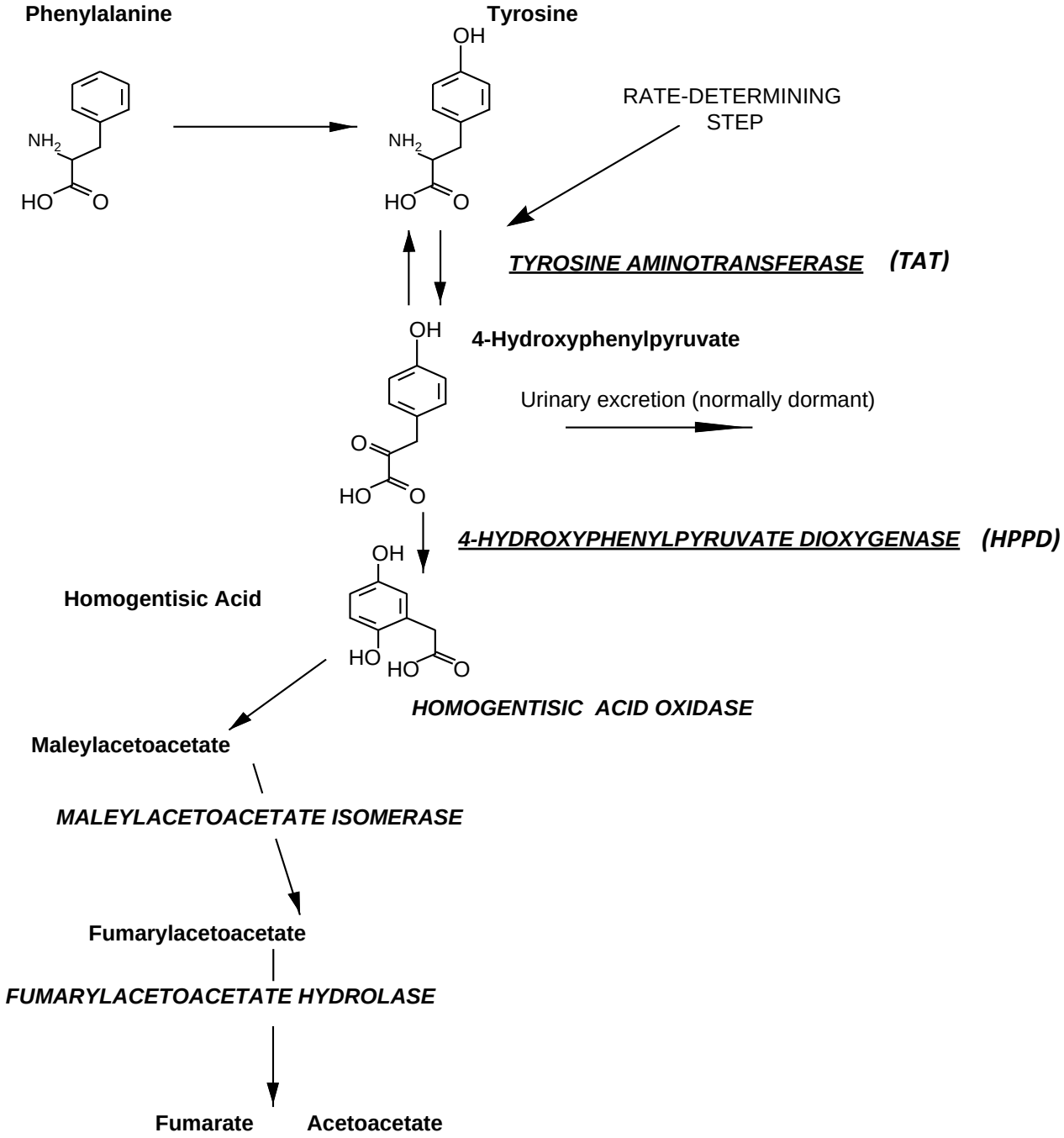
At the time of submission, the substance is not registered under REACH.

2.2 Short summary of the scientific justification for the CLH proposal

Mesotrione is a triketone herbicide and it exerts its Mode of Action (MoA) via inhibition of the enzyme 4- Hydroxyphenylpyruvate dioxygenase (HPPD) (Lee *et al.*, 1997). HPPD occurs in plants and animals, the 52 active site amino acid residues being similar across phyla and highly conserved within mammalian species. Direct measurement of hepatic and renal enzyme activity in rats and mice confirms that mesotrione inhibits HPPD and that this inhibition is reversible (Lock *et al.*, 1994).

In mammals, HPPD is the second enzyme in the catabolic cascade of tyrosine (figure 1):

Figure 1: Catabloic pathway of tyrosine



The consequence of HPPD inhibition is a dose- and species-dependent elevation in plasma tyrosine. If enzyme binding is reversible (as is the case with mesotrione) enzyme activity will recover and plasma tyrosine levels fall once exposure to mesotrione ceases.

Inhibition of HPPD leads to a build-up of its substrate 4-hydroxypheylpyruvic acid (4-HPPA), also known as 4-hydroxyphenylpyruvate, which is found in urine (Ellis *et al.*, 1995). The formation of HPPA from tyrosine by tyrosine aminotransferase (TAT) is reversible and a build-up of HPPA results in an elevation of tyrosine in the plasma (hypertyrosinaemia). Tyrosine aminotransferase (TAT), the first enzyme in the catabolic pathway, is the limiting and controlling enzyme of tyrosine catabolism. HPPD normally operates at a fraction of its maximum velocity (Lock *et al.*, 1996) and

tyrosine concentration is a function of the rate of formation/absorption of tyrosine, the activity of TAT and the efficiency of HPPA elimination by the kidney.

Innate hepatic TAT activity is higher in the mouse and a sex difference in the rat results in TAT activity in the female rat being higher than the male. The difference in TAT activity results in a species and sex difference in tyrosine accumulation.

Comparison of innate hepatic TAT (nmol/ HPPA/min/mg protein) activity – rats, mice and humans

	Rat	Mouse	Humans*
Male	1.7 ± 0.2	7.8 ± 1.5	7.17 ± 1.17
Female	3.3 ± 0.5	10.5 ± 1.9	

From Henderson *et al*., 1981

In the MoA proposed by the applicant, the spectrum of effects seen with mesotrione are said to be due to hypertyrosinaemia, rather than a direct result of dosing with mesotrione. Humans are expected to be less sensitive to the effects of mesotrione than rats, due to a higher innate TAT activity (for further information, see Annex 1).

Since the original decision regarding the classification and labelling of mesotrione, other HPPD-inhibitors (namely sulcotrione and tembotrione) have been considered by RAC. Both of these substances have been classified for repeated dose toxicity and reproductive toxicity.

In the repeated dose toxicity studies for sulcotrione, effects were noted on the cornea and kidney. RAC concluded that effects on the cornea (seen in rats and dogs) were caused by hypertyrosinaemia, and that due to species-specific differences in tyrosine catabolic pathways the rat, especially the male, is particularly sensitive to these effects. RAC used data on sulcotrione in monkeys, and data from studies on mesotrione, to conclude that the corneal effects resulting from administration of sulcotrione are not relevant to humans. With regards to the effects seen in the kidneys (see table below), RAC considered that classification with STOT RE 2 (kidneys) was appropriate. Sulcotrione was also classified as Repr. Cat 2, based on early pup deaths in rats.

Tembotrione was also found to cause effects on the cornea in rats, and the dossier submitter proposed hypertyrosinaemia as the cause. As part of the assessment of tembotrione, RAC reviewed the literature and concluded that hypertyrosinaemia is a relevant MoA in humans. The triketone analogue NTBC (2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione) is used as a pharmaceutical drug to inhibit HPPD, and the potency of tembotrione in humans was thought not to be that much lower than the potency of NTBC. As NTBC has been shown to greatly increase tyrosine concentrations in healthy adult volunteers treated with a single dose of 1 mg/kg/day NTBC, and to cause eye problems in some children treated with 1 mg/kg/day NTBC against hypertyrosinaemia type 1, tembotrione is expected to have an intrinsic possibility to also cause similar effects in humans. Concerning human sensitivity in relation to the animal data, the RAC concluded that this might be intermediate to that of the very sensitive rat and the non-sensitive mouse. RAC therefore considered that the findings in the rat studies were relevant to humans, and classified tembotrione as STOT RE Cat 2 for effects on the eyes (as well as the kidney and liver in rats, and premature deaths in rabbits).

Effects in the kidney (of rats) consisted of increased weight, histopathological findings consistent with chronic nephropathy (in males and females), and increased incidence of dilated renal pelvis. Effects in the liver (of rats) were generally mild, however fibrosis was noted at doses of >1 mg/kg bw/d. In a developmental study in rabbits, 5 out of 25 dams died prematurely in the 100 mg/kg bw/d

group. As the effect was not considered to be specific to pregnant rabbits, the mortality contributed to the classification as STOT RE 2.

Tembotrione was also classified in Repr Cat 2, based on skeletal developmental effects in rats (variations) and rabbits (anomalies and variations), and decreased pre- and postnatal growth rates in rats. RAC concluded that the skeletal effects seen in the rats were due to hypertyrosinaemia.

Classification of two other HPPD inhibitors (sulcotrione and tembotrione) for the STOT RE and reproductive toxicity end-points agreed by RAC, and summary of main effects.

Classification	**Effects**
Sulcotrione (RAC opinion dated 27th October 2011)	
STOT RE 2 (kidneys), H373	Effects in the kidneys: No significant effects in the sub-chronic studies Chronic studies (rats): increased weight, cystic kidneys, kidney enlargement, pelvis dilatation, calcification, papillary dilatation, tubule dilatation, increased severity of chronic progressive nephrosis Chronic studies (mice, high doses only): increased incidence and/or severity of papillary necrosis and calcification. Increased incidences of rough or pitted kidneys, pelvis and tubule dilatation. Reproductive toxicity studies (rats): pelvic dilatation, tubular basophilia, urinary tract abnormalities, small or misshaped kidneys
Repr. 2, H361d	Early pup deaths in rat two-generation studies (cause of deaths not known)
Tembotrione (RAC opinion dated 4th June 2013)	
STOT RE 2 (eyes, kidneys, liver), H373	Effects in the eyes (rats): Corneal opacities, neovascularisation and oedema of the cornea, snow flake-like corneal opacities, and keratitis Effects in the kidneys: No significant effects in the sub-chronic studies. Chronic studies (rats): increased weight, chronic nephropathy (tubular cell regeneration, thickened basement membranes, interstitial fibrosis, inflammation, dilated/cystic tubules, protein casts, pigmentation, mineralisation, debris, mesangial proliferation, glomerular sclerosis, and hypertrophy/hyperplasia of tubular epithelium Effects in the liver (rats): fibrosis Reproductive toxicity studies (rats): increased weight, increased incidence of dilated renal pelvis Developmental toxicity studies (rabbits): premature deaths in dams
Repr. 2, H361d	Developmental toxicity study (rats): reduced pup body weights, variations related to poor ossification Developmental toxicity study (rabbits): increased incidence of variations (extra ossification sites between atlas and axis centrum, incomplete ossification of pubis) and anomalies (presence of 27 presacral vertebrae in combination with 13 thoracic rib)

Regarding the environmental hazard classification proposal, this is discussed in detail in Section 5. In summary, technical mesotrione is determined to be 'not rapidly degradable' and not bioaccumulative. Fish and aquatic invertebrates are not acutely or chronically sensitive to mesotrione compared with algae and particularly higher aquatic plants/macrophytes. The most acutely sensitive species tested is the macrophyte *Lemna gibba*. It shows 7- to 14-day E_rC_{50} values of 0.0257 to 0.028 mg mesotrione/L, these are <0.1 mg/L and are in the range >0.01 to <0.1 mg/L, therefore mesotrione should be classified according to CLP criteria as: 'Aquatic Acute Category 1' (H400) with an Acute M-factor of 10.

The most chronically sensitive species tested is also *Lemna gibba* with 7- to 14-day NOE_rC values of 0.002 mg mesotrione/L. These are also supported by an E_rC_{10} value of 0.002 mg/L. These endpoints are between >0.001 and <0.01 mg/L and, since mesotrione is considered 'not rapidly biodegradable', it should be classified as: 'Aquatic Chronic Category 1' (H410) with a Chronic M-factor of 10.

2.3 Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

Classification		Labelling	
Hazard Class and Category Code(s)	**Hazard Statement Code(s)**	**Hazard Statement Code(s)**	**Pictograms, Signal Word Code(s)**
Aquatic Acute 1	H400		GHS09
Aquatic Chronic 1	H410	H410	Warning

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling

At the time of submission there are 178 notifications to the C&L inventory for mesotrione. All notifiers have notifed the same classification which is in line with the current harmonised classification and labelling in Annex VI of the CLP Regulation.

Classification		Labelling	
Hazard Class and Category Code(s)	**Hazard Statement Code(s)**	**Hazard Statement Code(s)**	**Pictograms, Signal Word Code(s)**
Aquatic Acute 1	H400		GHS09
Aquatic Chronic 1	H410	H410	Warning

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Mesotrione is a pesticide active substance subject to renewal under Regulation (EC) 1107/2009, for which the UK is the Rapporteur Member State (RMS). The substance has an existing entry in Annex VI of CLP which includes classification for environmental hazards only. Due to concerns raised during the EFSA peer review process, a targeted review of the classification and labelling is considered appropriate. This proposal therefore addresses only the following hazard classes: STOT-RE, mutagenicity, carcinogenicity, reproductive toxicity and hazardous to the aquatic environment.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

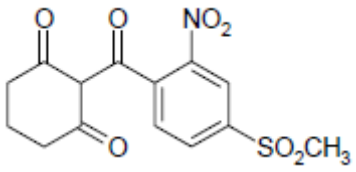
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 4: Substance identity

EC number:	Not listed
EC name:	Not listed
CAS number (EC inventory):	Not listed
CAS number:	104206-82-8
CAS name:	1,3-Cyclohexanedione, 2-[4-(methylsufonyl)-2-nitrobenzoyl]-
IUPAC name:	2-(4-mesyl-2-nitrobenzoyl) cyclohexane -1,3-dione
CLP Annex VI Index number:	609-064-00-X
Molecular formula:	$C_{14}H_{13}NO_7S$
Molecular weight range:	339.3

Structural formula:



1.2 Composition of the substance

Table 5: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Mesotrione	≥ 93%		

Current Annex VI entry:

Table 6: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
1,2-dichlorethane CAS 107-06-2 EC 203-458-1	< 0.1%	< 0.1%	Current Annex VI entry: Flam. Liq 2; H225 – Highly flammable liquid and vapour Acute Tox 4; H302 – Harmful if swallowed Skin Irrit.2 ; H315 – Causes skin irritation Eye Irrit.2; H319 – Causes serious eye irritation STOT SE 3; H335 – May cause respiratory irritation Carc. 1B; H350 – May cause cancer

There are a number of process impurities in the substance. Three were identified as relevant impurities in the EFSA conclusion, including 1,2-dichloroethane which has an existing harmonised entry in Annex VI of CLP as provided in table 6. All impurities have been taken into account and, considering the concentration at which they are present and the data available on mesotrione, they are not considered to impact on the classification proposed in this dossier. Further information on the impurities is considered to be confidential but full details are provided in the IUCLID.

It is noted that for a substance classified as Carc 1B; H350, the generic concentration limit that will trigger classification of a mixture (or another substance where present as an impurity) as a carcinogen in ≥ 0.1%. Therefore, should the concentration of 1,2-dichloroethane be ≥ 0.1% this should be taken into account in the classification.

Table 7: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
None				

1.2.1 Composition of test material

The purity of mesotrione tested in the studies ranged from 92% - 98.1%. Where purity was given as 83% (immunotoxicity study) the lower percentage purity was attributable to the water content of this technical wet paste. Information on the actual purity used is provided in the relevant summaries and tables of this report. The tested material in all cases is considered to be equivalent to and representative of that specified above.

Note; in some of the study summaries the technical material is referred to as 'ZA 1296' which was a development code for mesotrione.

1.3 Physico-chemical properties

Table 8: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	Pale yellow solid at room temperature	Goodman, M (1996a) RAR B.2.3	pure material, 99.3% w/w
	Light tan or sand coloured opaque solid	Goodman, M (1996b) RAR B.2.3	technical material, 96.7% w/w
	Pale amber/brown solid in the form of a soft, granular powder, containing coarse particles and some larger aggregates	O'Connor, B. J. (2013 a) RAR B.2.3	technical material (wet paste), 83.0 % w/w
Melting/freezing point	Melting point: 165.3°C with decomposition	Goodman, M (1996a) RAR B.2.1	EEC Method A1 Decomposition evidenced by dark red colour and vapour evolution
Boiling point	The test substance decomposes without boiling ≈ 175 °C	Vehling, H. (2005) RAR B.2.1	EEC Method A2 (DSC) pure material, 99.6% w/w
Vapour pressure	$<5.7 \times 10^{-6}$ Pa at 20°C This value was taken as the maximum on the basis of the vapour pressures observed: $<3.3 \times 10^{-6}$ at 50.7 ºC $<5.7 \times 10^{-6}$ at 100.7 ºC 2.2×10^{-5} at 110.2ºC	Goodman, M (1996a) Bartley, G (1999e) RAR B.2.2	EEC Method A4 pure material, 99.7% w/w Essentially non volatile
	4.7×10^{-7} Pa at 20°C	Goodman, M (1997a) RAR B.2.2	EEC Method A4 AMBA*, 99% w/w Essentially non volatile
	6.4×10^{-7} Pa at 20°C	Goodman, M (1997b) RAR B.2.2	EEC Method A4 MNBA*, 99.6% w/w Essentially non volatile

Surface tension	Aqueous solution, 90% saturated with mesotrione: 72.5 mN/m at 20°C which indicates that mesotrione is not surface active	Goodman, M (1996b) RAR B.2.12	EEC Method A5 technical material, 96.7% w/w
	Aqueous solution, 90% saturated with mesotrione: 61.5 – 61.6 mN/m at 21.0 ± 0.5 °C which indicates that mesotrione is not surface active	O'Connor, B. J. (2013 b) RAR B.2.12	OECD Test guideline 115 and EC Method A5 pure material, 99.5% w/w
Water solubility	at 20°C: 160 mg/L in unbuffered water 2200 mg/L at pH 4.8 1500 mg/L at pH 6.9 2200 mg/L at pH 9	Goodman, M (1996a) RAR B.2.5	EEC Method A6 pure material, 99.7% w/w Moderately soluble in unbuffered water
	at 20°C: 300 mg/mL in unbuffered water 1800 mg/L at pH 4.7 2300 mg/L at pH 6.1	Goodman, M (1997a) RAR B.2.5	Erlenmeyer flasks were used in line with EEC Method A6 AMBA, 99% w/w Moderately soluble in unbuffered water
	at 20°C: 4000 mg/L in unbuffered water 14,200 mg/L at pH 2.6 32,400 mg/L at pH 2.9 Attempts to maintain higher pHs were unsuccessful	Goodman, M (1997b) RAR B.2.5	Erlenmeyer flasks were used in line with EEC Method A6 MNBA, 99.6% w/w Readily soluble in unbuffered water

Partition coefficient n-octanol/water	At 20ºC log P_{ow}: 0.11 in unbuffered water -1.1 at pH 5 <-1.0 at pH 7 and 9	Goodman, M (1996a) RAR B.2.7	EEC Method A8 pure material, 99.7% w/w Not lipophilic
	At 20°C log P_{ow}: 0.32 in unbuffered water -0.30 in pH 5 buffered water	Goodman, M (1997a) RAR B.2.7	EEC Method A8 AMBA, 99% w/w Not lipophilic
	At 20°C log P_{ow}: -1.3 in unbuffered water -2.6 in pH 5 buffered water	Goodman, M (1997b) RAR B.2.7	EEC Method A8 MNBA, 99.6% w/w Not lipophilic
Flammability	Mesotrione did not propagate combustion and is therefore not classified as highly flammable in terms of burning	Goodman, M (1996b) RAR B.2.9	EEC Method A10 technical material, 96.7% w/w
Explosive properties	Mesotrione technical is not classified as an explosive in terms of its mechanical, frictional or thermal sensitivity	Goodman, M (1996b) RAR B.2.11	EEC Method A14 technical material, 96.7% w/w
Oxidising properties	Mesotrione is not classified as an oxidising agent	Goodman, M (1996b) RAR B.2.13	EEC Method A17 technical material, 96.7% w/w
Dissociation constant	pka 3.12 at 20°C. Absorbance of the completely associated and unassocicated mesotrione was 0.6503 and 0.3402 m^{-1} cm^{-1} (molar extinction coefficient), respectively at 254nm.	Goodman, M (1996a) RAR B.2.8	OECD Test guideline 112 pure material, 99.7% w/w

2 MANUFACTURE AND USES

2.1 Manufacture

Mestotrione is manufactured both inside and outside of the EU.

2.2 Identified uses

Mesotrione is a systemic herbicide to control most annual broadleaf and annual grass weed.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Physico-chemical end points are not considered in this classification and labelling proposal.

Table 9: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
Refer to table 8			

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

This brief summary is based on the information in the Renewal Assessment Report (RAR – Mesotrione – Volume 3, Annex B.6; Toxicology and Metabolism – February 2015).

4.1.1 Non-human information

The adsorption, distribution, metabolism and excretion of mesotrione have been investigated in rats and mice following single and multiple oral gavage doses, and single intravenous dosing.

Absorption

Mesotrione is rapidly and extensively absorbed in rats and mice following oral administration.

In rats, systemic absorption following a single oral dose of 1 mg [^{14}C]-mesotrione / bw kg was estimated by comparison of the urinary excretion (including cage wash) corrected for the recovery of administered radioactivity with that from rats given a similar intravenous dose. The estimated absorption values ranged from 62.2 – 72.6% in males and 68.3 – 72.6% in females. The mean peak blood concentration (C_{max}) of radioactivity occurred 0.5 hours after dosing in each sex

Absorption following a single oral dose of 100 mg/kg was calculated as 67% for males and 69% for females from the sum of radioactivity measured in urine, cage-wash and tissues after correction for the recovery of administered radioactivity The mean peak blood concentration (C_{max}) of radioactivity following a single oral dose of 100 mg [^{14}C]-mesotrione / bw kg occurred 1.5 hours after dosing in each sex.

In mice, systemic absorption following single oral dose of 1 mg/kg was 39.2 – 44% in males and 61.7 – 62.9% in females. Absorption following a single oral dose of 100 mg/kg was calculated as 70% for males and 74% for females from the sum of radioactivity measured in urine, cage-wash and tissues after correction for the recovery of administered radioactivity. Peak blood concentrations (C_{max}) occurred approximately 1 hour after dosing in both sexes and at both doses.

Based on the available rodent data, an absorption value of 70% is identified in the RAR.

Distribution

In a whole body autoradiography study in the rat, 24 hours after a single dose of mesotrione (5 mg [^{14}C]-mesotrione/kg bw) the greatest intensity of radiolabelling was present in the contents of the gastrointestinal (GI) tract, which is consistent with the observed faecal excretion of radioactivity. Radiolabelled residues were apparent in both sexes in the liver and kidney. No significant residues were observed in any other tissues. At 48 hours after dosing, radioactivity had declined in the GI tract but remained in the liver and kidneys. There were no marked differences in the relative intensity of radioactivity in tissues between the sexes.

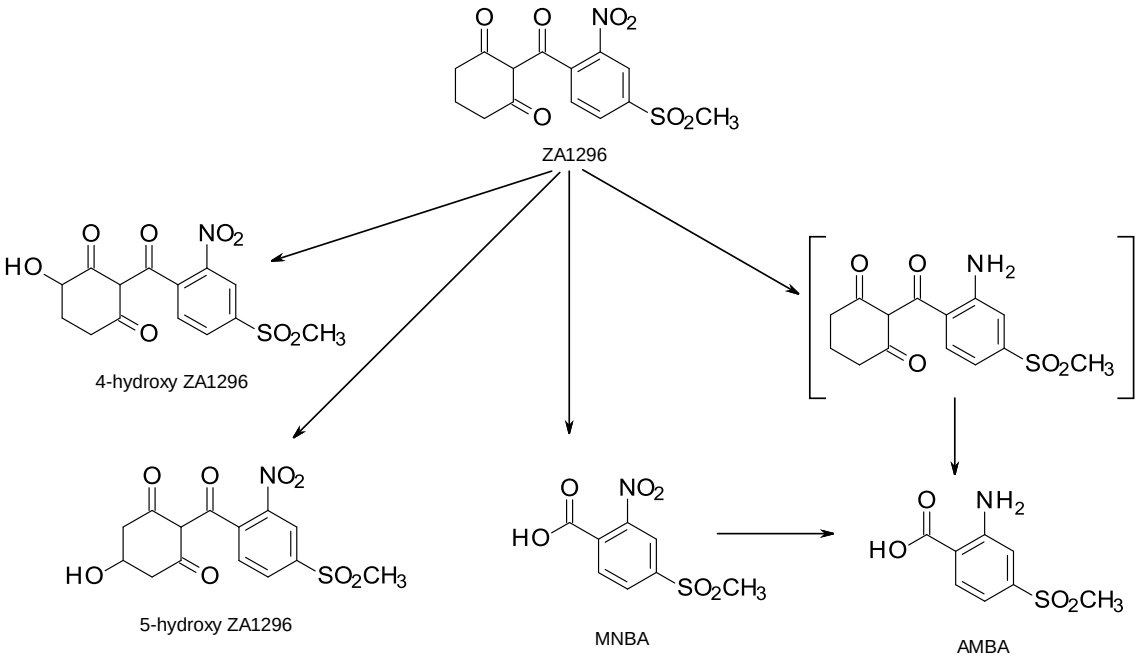
The data from the whole body autoradiography were consistent with the observations from excretion and distribution studies. For rats administered 1mg [^{14}C]-mesotrione/kg bw, the liver contained 12 and 9% and the kidneys 0.3 and 0.9% of the administered dose for males and females respectively (72 hours after dosing), which together accounted for over 90% of the radioactivity present in the carcass. In another oral dosing study, similar results of 9 and 7% in the liver and 0.2 and 0.7% in kidneys for males and females respectively were observed 7 days after dosing, and closely matched those obtained from rats following a similar single intravenous dose of 1 mg [^{14}C]-

mesotrione/kg bw. After a dose of 100 mg mesotrione/kg, the liver contained 0.2% for both sexes, and kidneys 0.008 and 0.013% of the dose for males and females, respectively, that together accounted for 33 and 17% of the radioactivity present in the carcass. Whereas the hepatic concentrations were similar between the sexes, the renal concentrations were higher in females than in males. It is notable that despite the 100-fold differential in dose, the hepatic concentrations were only twice those observed after the lower dose and the renal concentrations were less than 3 times higher. Tissue concentrations were very low in all other tissues analysed. A similar distribution of radioactive residues in the rat was seen after repeat dosing.

Tissue distribution in mice was similar to that seen in the rat. The highest tissue concentrations in both sexes were present in the liver and kidneys, which together accounted for over 94% of the radioactivity present in the carcass at the termination of the 1 mg/kg dose studies. 168 hours after dosing, the liver contained 6.8% of the administered dose in males and 8.25% of the dose in females. With the exception of the kidneys that represented 0.05% of the dose in males and 0.5% in females, all other tissues contained negligible amounts. A comparison of tissue concentrations between the 1 and 100 mg/kg dose levels showed a substantial differential for all tissues with the single exception of the liver.

The elevated levels of radioactivity measured in the liver observed in both rats and mice at the lower dose may be due to the binding of mesotrione to the HPPD enzyme which is the target for this class of herbicides and is located in the liver. Furthermore, comparisons of the results from the different doses used in the studies suggest that the capacity for liver to retain mesotrione is saturable.

Metabolism



Structure in square brackets indicates a postulated intermediate

In biotransformation studies, the quantitative and qualitative nature of metabolites formed has been determined. Biliary elimination and biotransformation studies using [^{14}C-dione]- or [^{14}C-aromatic]-radiolabelled mesotrione indicated that there were no pronounced differences in the metabolism of these two labelled forms of the compound and thus very little cleavage of the carbonyl bridge occurred between the cyclohexadione and phenyl moieties. Similarly, in a quantitative whole-body autoradiography (QWBA) study there were no clear differences in tissue distribution profiles between the [^{14}C-dione]- or [^{14}C-aromatic]- mesotrione.

Mesotrione was not extensively metabolised in rats or mice since most of the absorbed dose was excreted unchanged in urine (*ca.* 90% or more of the urinary radioactivity) independent of dose or route. There was a quantitative sex difference apparent in the metabolism of absorbed mesotrione with male rats transforming a higher proportion of parent compound into metabolites than females. Small amounts of minor metabolites including 4-hydroxy mesotrione, 5-hydroxy mesotrione, MNBA and AMBA were excreted in rat urine. Mesotrione was also eliminated in bile, this being more pronounced in male rats. The 4-hydroxy metabolite was the only other identified radioactive component in bile and this was present in male rats only. However there was evidence of metabolism of mesotrione by the intestinal flora, resulting in an array of rat faecal metabolites, including cleavage between the aromatic and dione rings to give MNBA and AMBA. These metabolites appear to have been reabsorbed and excreted in urine. This is supported by a study conducted in rats administered a single oral dose of 75 mg [^{14}C]-MNBA/kg bw. MNBA was reduced to AMBA in the gastrointestinal tract and excreted in urine. There were no differences in the metabolite profiles of rats given a single oral dose or 14 consecutive daily doses of mesotrione.

In mice, a small amount of hydroxy mesotrione (1%) was detected in urine from males together with AMBA (1%) and trace amounts of MNBA following the administration of ^{14}C-mesotrione. Small amounts of MNBA and AMBA were also measured in mouse faecal samples.

The biotransformation of mesotrione in rats and mice was shown to be limited with small amounts of hydroxy mesotrione as the principle metabolite in rat. This is consistent with the metabolism observed in humans (see section 4.1.2) where a large proportion of the dose administered to volunteers was excreted in urine as mesotrione.

Excretion

In rats administered a single oral dose of 1 or 100 mg [^{14}C]-mesotrione/kg bw, most (over 70%) of the radioactivity was eliminated within 24 hours of dosing. Renal elimination represented the principal route of excretion independent of sex, dose or dose route, accounting for over 45% of the total corrected for recovery. At 72 hours post dose, the total amounts of radioactivity excreted in urine and faeces for both male and female rats were 78-98% of the dosed radioactivity, the gastrointestinal tract contents containing less than 0.1-0.2% of the dose in both sexes. The total mean percentage recoveries, including excreta, tissues and carcass, were 92-96% of the administered dose. Biliary excretion measured in bile duct cannulated rats following oral administration was more extensive in males (10-14%) than in females (2-4%). Faecal excretion of less than 7% in males and less than 3% in females of the dosed radioactivity following intravenous administration to rats corresponded well with the proportion of the dose excreted in the bile and implied that biliary elimination was limited. The radioactive residues in expired air was <1%. Comparison of the relative proportions of radioactivity excreted in urine and faeces with those obtained from a single oral dose indicated a slightly increased initial rate of excretion of radioactivity in urine following the repeated administration of mesotrione. The presence of radioactivity in expired air was negligible, accounting for less than 0.1% of administered radioactivity over 24 hours.

A similar excretion profile was observed in the mouse with most (over 70%) of the radioactivity being excreted within the first 24 hours after dosing for male and female mice administered a single oral dose of 1 or 100 mg [^{14}C]-mesotrione/kg bw. Taking account of the variability in individual animal data, it was apparent that for both sexes, the dose was excreted predominantly in urine and with a greater proportion excreted in urine from female mice compared to males. At study termination, the gastrointestinal tract contained less than 0.1% of the dose for both sexes. The mean total percentage recoveries, including excreta, tissues and carcass, for male and female mice, were 91 to 95% of the administered dose. The amount of radioactivity recovered as exhaled volatile metabolites after a 1 mg/kg bw dose level was negligible.

4.1.2 Human information

See Section 4.7.1.6

4.1.3 Summary and discussion on toxicokinetics

The toxicokinetics of mesotrione has been assessed in studies investigating the absorption, distribution, metabolism and excretion of the chemical in rats and mice following single and multiple oral gavage and single intravenous dosing. Mesotrione was well absorbed with systemic absorption measured as ca. 70% of the administered radioactivity following a single oral dose. The majority of absorbed mesotrione was excreted via the urine. Mesotrione is not extensively metabolised in the rat or mouse and is predominantly excreted unchanged in the urine (ca. 90% of the urinary radioactivity). Small amounts of 4-hydroxy mesotrione, 5-hydroxy mesotrione, MNBA and AMBA were excreted in rat and mouse urine. MNBA and AMBA are produced by intestinal flora and can be reabsorbed and excreted in urine.

In a human volunteer study (see Section 4.7.1.6), the toxicokinetics of mesotrione in humans were similar to those in mice and rats. Mesotrione was rapidly absorbed (peak plasma concentrations within 2 hours of dosing) and rapidly excreted unchanged in the urine (majority of the recovered dose was excreted within 12 hours of dosing).

4.2 Acute toxicity

This hazard class is not addressed in this CLH proposal.

4.3 Specific target organ toxicity – single exposure (STOT SE)

This hazard class is not addressed in this CLH proposal.

4.4 Irritation

4.4.1 Skin irritation

This hazard class is not addressed in this CLH proposal.

4.4.2 Eye irritation

This hazard class is not addressed in this CLH proposal.

4.4.3 Respiratory tract irritation

This hazard class is not addressed in this CLH proposal.

4.5 Corrosivity

This hazard class is not addressed in this CLH proposal.

4.6 Sensitisation

4.6.1 Skin sensititsation

This hazard class is not addressed in this CLH proposal.

4.6.2 Respiratory sensitisation

This hazard class is not addressed in this CLH proposal.

4.7 Repeated dose toxicity

4.7.1 Non-human information

The repeated dose toxicity of mesotrione has been investigated by the oral route (in rats, mice and dogs) and by the dermal route in rabbits. No data are available via the inhalation route.

4.7.1.1 Repeated dose toxicity: oral

i) Repeated dose toxicity by the oral route in rats

The following studies are available to investigate the repeated dose toxicity of mesotrione in the rat via the oral route:

1. *28-day study (OECD 407) with dose levels 0, 1000, 5000, 10000, 15000 and 20000 ppm mesotrione.*
2. *90-day study (OECD 408) with dose levels 0, 1, 125, 1250 and 12500 ppm mesotrione.*
3. *90-day study (OECD 408) with dose levels 0, 2.5, 5, 7.5 and 150 ppm mesotrione.*
4. *90-day study (non-guideline) with dose levels 0, 5, 100 or 2500 ppm mesotrione (study to investigate reversibility of effects).*
5. *90-day study (non-guideline) with dose levels 0, 0.5, 1.3, 4, 5, 7.5, 10 or 100 ppm mesotrione (study to investigate response, male rats only)*
6. *90-day study (non-guideline) with dose levels 0, 1, 5, 10, 50, 100, 1000 or 2500 ppm mesotrione (study to investigate response, female rats only)*
7. *90-day study (non-guideline) with dose levels 0, 10, 20, 50 or 125 ppm mesotrione (study to investigate ocular end points)*
8. *Ocular toxicity development and reversibility study (non-guideline) with dose levels 0 or 2500 ppm mesotrione*
9. *2-year dietary toxicity and oncogenicity study (OECD 453) with dose levels 0, 1, 2.5, 7.5, 100 and 2500 ppm mesotrione*

The studies are summarised in table 10 and further information is presented after the table.

Three additional (non-guideline) studies are available which provide further information on the effects seen in rats following repeated exposure to mesotrione or tyrosine. These include:

1. *21-day study to investigate effects of dietary tyrosine on ocular effects – animals dosed with 0, 0.5, 1.0, 2.5 and 5.0% tyrosine in a low protein diet.*
2. *28-day study to investigate ocular effects, plasma tyrosine concentration and enzyme (HPPD and TAT) activity – dynamic dosing (0 or variable dietary levels of mesotrione).*
3. *28-day study with varying combinations of mesotrione and/or tyrosine, investigating ocular effects, plasma tyrosine concentration and enzyme (HPPD and TAT) activity.*

The studies are summarised in table 11.

Further information on repeated dose toxicity in rats is provided by a guideline multigeneration study and a developmental toxicity study (see sections 4.11.1 and 4.11.2 respectively). The relevant results are discussed in the summary section for repeated dose effects (section 4.8.1).

All studies were considered to be acceptable and reliable for classification and labelling purposes.

Table 10: Summary table of repeated dose toxicity studies by the oral route in rats

↑/↓ = increased/decreased compared to control. Unless otherwise stated, effects were seen in both sexes.
*significantly different to controls ($p<0.05$), ** $p<0.01$
#The values for the NOAELs are provided for information only. All NOAEL values provided in this CLH Report have been taken from the Renewal Assessment Report (RAR), produced in accordance with Directive 91/414/EEC.

Method	Dose Levels	Significant Toxicological Findings
28 day oral study OECD 407 (minor deviations that do not effect reliability) GLP compliant Alpk:AP$_f$SD rats, 6/sex/dose Mesotrione (>98.1%) in diet Anonymous (1994a) RAR B.6.3.1 **In a standard 28 day study in the rat, effects seen at doses ≤ 300 mg/kg bw/d are relevant for classification for repeated dose toxicity.**	0, 1000, 5000, 10000, 15000 and 20000 ppm Equivalent to: 0, 131, 656, 1315, 1871 and 2464 mg/kg bw/day in males and 0, 133, 651, 1324, 1916 and 2424 mg/kg bw in females	**1000 ppm (131 / 133 mg/kg bw/ day)** *Urinalysis* ↑ urine specific gravity (females) Blood in urine (1/6 males) *Kidney* Hyaline droplets (6/6 males, 3/6 females, cf. 0/6 and 0/6 in controls) Transitional epithelial hyperplasia (2/6 males, cf. 0 in controls **5000 ppm (656 / 651 mg/kg bw/day)** *Clinical chemistry* ↑ plasma creatinine levels (females 14%) *Urinalysis* Blood in urine (3/6 males) *Kidney* Hyaline droplets (6/6 males, 6/6 females) Transitional epithelial hyperplasia (1/6 males, cf. 0 in controls) **10000 ppm (1315 / 1324 mg/kg bw/d)** *Body weight* ↓ mean body weight (males, 9.8%) *Clinical chemistry* ↑ plasma creatinine levels (males 19%, females 10%) ↑ bilirubin (males 39%) ↑ APDM activity (males 36%, females 40%) *Urinalysis* ↓ urine pH (males) ↑ urine specific gravity (females) Blood in urine (5/6 males) *Kidney* Hyaline droplets (6/6 males, 6/6 females) Transitional epithelial hyperplasia (2/6 males, 1/6 females cf. 0 in controls *Liver* ↑ relative weight (females 10.9%)

		15000 ppm (1871 / 1916 mg/kg bw/day) *Body weight* ↓ mean body weight (males 12.1%, females 10.4%) *Clinical chemistry* ↑ plasma creatinine levels (males 14%, females 20%) ↑ bilirubin (males 56%) ↓ alkaline phosphatase activity (males, 13%) ↑ APDM activity (males 46%, females 35%) *Urinalysis* ↓ urine pH (males) ↑ urine specific gravity (females) Blood in urine (2/6 males) *Kidney* Hyaline droplets (6/6 males, 6/6 females) Transitional epithelial hyperplasia (1/6 males) *Liver* ↑ relative weight (females 15.2%) **20000 ppm (2464 / 2424 mg/kg bw/day)** *Body weight* ↓ mean body weight (males 19.8%, females 10.1%) *Clinical chemistry* ↑ plasma creatinine levels (males 23%, females 24%) ↑ cholesterol (males, 23%) ↑ bilirubin (males 39%) ↓ alkaline phosphatase activity (males, 11%) ↑ APDM activity (males 44%, females 42%) *Urinalysis* ↓ urine pH (males) Blood in urine (3/6 males) *Kidney* Hyaline droplets (6/6 males, 4/6 females) Transitional epithelial hyperplasia (1/6 males) ↑ renal pelvic dilatation (4/6 males, cf. 1/6 controls) ↑ hydronephrosis (3/6 males cf. 2/6 controls; 2/6 females, cf. 0/6 controls) *Liver* ↑ relative weight (females 9.8%) *A #NOAEL could not be identified in this study due to renal microscopic findings at all dose levels.*

90 day oral study OECD 408 (minor deviations that do not effect reliability) GLP compliant Alpk:AP$_f$SD rats, 12/sex/dose Mesotrione (93.3% purity) in diet Anonymous (1994b) RAR B.6.3.2 **In a standard 90 day study in the rat, effects seen at doses ≤ 100 mg/kg bw/d are relevant for classification for repeated dose toxicity.**	0, 1, 125, 1250 and 12500 ppm Equivalent to 0, 0.09, 11.0, 112, 1111 mg/kg bw/d in males and 0, 0.1, 12.8, 126 and 1213 mg/kg bw/d in females	**1 ppm (0.09 / 0.1 mg/kg bw/d)** No adverse effects **125 ppm (11 / 12.8 mg/kg bw/d)** *Eyes* Corneal opacity, keratitis and vascularisation (see table below) *Clinical chemistry* ↑ creatinine (19% males, 10.8% females) ↑ triglycerides (females 29.5%) *Kidneys* ↑ relative kidney weight (males, 9.5%) Hyaline droplets (4/12 males, cf. 0/12 in controls) *Liver* ↑ relative liver weight (16.7% males, 10.5% females) **1250 ppm (112 / 126 mg/kg bw/d)** *Eyes* Corneal opacity, keratitis and vascularisation (see table below) *Clinical chemistry & urinalysis* ↑ creatinine (18% males, 20% females) ↑ triglycerides (females 48%) ↓ urine pH (males) *Kidneys* ↑ relative kidney weight (13.8% males) *Liver* ↑ relative liver weight (17.8% males, 12.6% females) **12500 ppm (1111 / 1213 mg/kg bw/d)** *Eyes* Corneal opacity, keratitis, vascularisation and epithelial disruption (see table below). *Body weight* ↓ body weight (15.3% males, 9.4% females) ↓ food utilization (12.6% males, 6.2% females) *Clinical chemistry & urinalysis* ↑ creatinine (29% males, 29% females) ↑ triglycerides (females 57%) ↓ urine pH ↑ urine protein (females) *Kidneys* ↑ relative kidney weight (10.9%, males) Hyaline droplets (11/12 males, cf. 0/12 in controls) *Liver* ↑ relative liver weight (17.8% males, 12.6% females)

Ocular findings (all doses):

	Dose Group (ppm)				
	0	**1**	**125**	**1250**	**12500**
	Males				
Ophthalmoscopy findings in the cornea					
Degree of corneal opacity:					
Hazy/ slight	0	0	0	0	0
Hazy/ moderate	0	0	2	2	0
Hazy/ marked	0	0	8	9	5
Opaque/slight	0	0	0	0	1
Opaque/moderate	0	0	0	0	2
Opaque/ marked	0	0	1	1	0
Vascularisation	0	0	10	10	6
Microscopy findings					
Corneal keratitis	0	0	10	9	7
Vascularisation	0	0	9	9	7
Epithelial disruption	0	0	0	0	1
	Females				
Ophthalmoscopy findings in the cornea					
Degree of corneal opacity:					
Hazy/ slight	0	0	0	1	1
Hazy/ moderate	0	0	0	2	4
Hazy/ marked	0	0	0	3	8
Opaque/slight	0	0	1	4	1
Opaque/moderate	0	0	0	2	0
Opaque/ marked	0	0	0	0	0
Vascularisation	0	0	0	4	9
Microscopy findings					
Corneal keratitis	0	0	1	5	9
Vascularisation	0	0	0	7	8
Epithelial disruption	0	0	0	1	0

#NOAEL = 1 ppm (equivalent to 0.09 and 0.10 mg/kg bw/d

90 day oral study OECD 408 (minor deviations that do not effect reliability) GLP compliant Alpk:AP$_f$SD rats, 12/sex/dose Mesotrione (96.8% purity) in diet **In a standard 90 day study in the rat, effects seen at doses ≤ 100 mg/kg bw/d are relevant for classification for repeated dose toxicity.** Anonymous (1997a) RAR B 6.3.2.1	0, 2.5, 5.0, 7.5 and 150 ppm Equivalent to 0, 0.23, 0.47, 0.71 and 14.5 mg/kg bw/d in males and 0, 0.21, 0.41, 0.63 and 12.5 mg/kg bw/d in females	For ocular findings, see table below. Other findings (by dose): **2.5 ppm (0.23 / 0.21 mg/kg bw/d)** *Urinalysis* ↑ incidence of protein in urine 6/12 females cf. 2/12 controls ↑ incidence of ketones in urine, 2/12 males cf. 0/12 controls, and 3/12 females cf. 1/12 controls) *Kidneys* Renal pelvic dilatation (1/12 males, cf. 0/12 controls) **5.0 ppm (0.47 / 0.41 mg/kg bw/d)** *Urinalysis* ↑ incidence of protein in urine 5/12 males cf. 1/12 in controls, and 4/12 females cf. 2/12 controls ↑ incidence of ketones in urine, 2/12 males cf. 0/12 controls, and 2/12 females cf. 1/12 controls) *Liver* ↑ relative weight (males 12.7%) *Kidneys* Renal pelvic dilatation (2/12 males, cf. 0/12 controls) **7.5 ppm (0.71 / 0.63 mg/kg bw/d)** *Clinical chemistry & urinalysis* ↑ cholesterol (males, 15%) ↑ incidence of protein in urine 5/12 males cf. 1/12 in controls, and 4/12 females cf. 2/12 controls ↑ incidence of ketones in urine, 5/12 males cf. 0/12 controls, and 3/12 females cf. 1/12 controls *Liver* ↑ relative weight (males 16.0%) *Kidneys* Renal pelvic dilatation (1/12 males, cf. 0/12 controls) **150 ppm (14.5 / 12.5 mg/kg bw/d)** *Haematology, clinical chemistry & urinalysis* ↑ LUC (33.0% males, 47.9% females) ↑ cholesterol (14.4%, females) ↓ AP (17%, males) ↑ ALT (39%, males) ↑ AST (53%, males) ↑ CK (587.2%, females) ↑ potassium (14%, females) ↑ incidence of protein in urine 4/12 males cf. 1/12 in controls ↑ incidence of ketones in urine, 11/12 males cf. 0/12 controls, and 10/12 females cf. 1/12 controls)

Kidneys
Renal pelvic dilatation (1/12 males, cf. 0/12 in controls)
Hydronephrosis (3/12 males, cf. 1/12 controls)
Chronic progressive glomerulonephropathy (2/12 males, cf. 0/12 controls)
Liver
↑ relative weight (males 17.7%, females 10.0%)

Ocular findings (all doses):

	Dose Group (ppm)				
	0	**2.5**	**5.0**	**7.5**	**150**
	Males				
Ophthalmoscopy findings in the cornea					
Degree of corneal opacity:					
Hazy/ slight	1	0	0	1	0
Hazy/ moderate	1	0	0	0	0
Hazy/ marked	0	0	0	0	5
Opaque/slight	0	0	1	3	1
Opaque/moderate	0	0	0	3	2
Opaque/ marked	0	0	0	0	1
Vascularisation	0	0	0	3	8
Microscopy findings					
Corneal keratitis	0	0	0	4	7
	Females				
Ophthalmoscopy findings in the cornea					
Degree of corneal opacity:					
Hazy/ slight	0	0	0	0	0
Hazy/ moderate	0	0	0	0	0
Hazy/ marked	0	0	0	0	0
Opaque/slight	0	0	0	0	5
Opaque/moderate	0	0	0	0	0
Opaque/ marked	0	0	0	0	0
Vascularisation	0	0	0	0	0
Microscopy findings					
Corneal keratitis	0	0	0	0	1

#NOAEL = 2.5 ppm (equivalent to 0.24 mg/kg bw/d) based on increased liver weight in males at ≥ 5ppm

90 day reversibility studies Non-guideline GLP compliant Alpk:AP_fSD rats, males only (40/dose) Mesotrione (96.8% purity) in the diet Anonymous (1997b) RAR B.6.8.2.6	0, 5, 100 or 2500 ppm Equivalent to 0, 0.37, 7.5 and 192 mg/kg bw/d	For Ophthalmoscopy, biochemical and plasma tyrosine findings, see table below. Other effects (by dose): **5 ppm (0.37 mg/kg bw/d)** *Clinical signs* Cloudy eyes (1/40, cf. 0/40 in controls) *Kidneys* ↑ weight (11.7%, recovered after 2 weeks) *Liver* ↑ weight (9.6%, recovered after 2 weeks) **100 ppm (7.5 mg/kg bw/d)** *Clinical signs* Cloudy eyes (32/40, cf. 0/40 in controls) *Kidneys* ↑ weight (11.7%, recovered after 2 weeks) *Liver* ↑ weight (13.3%, recovered after 2 weeks) **2500 ppm (192 mg/kg bw/d)** *Clinical signs* Cloudy eyes (33/40, cf. 0/40 in controls) *Kidneys* ↑ weight (10.5%, recovered after 2 weeks) *Liver* ↑ weight (19.8% after 90 days of treatment, 10.1% after 9 weeks recovery) Ophthalmoscopy findings

Dose (ppm)	**Time (week)**	**No. eyes**	**Hazy**				**Opaque**				**Vascularis-ation**	
			+/-	+	++	+++	+/-	+	++	+++		**ghost**
5	14	80	--	--	--	--	2	7	1	--	2	--
	+2	16	--	--	--	--	--	--	--	--	--	--
	+4	16	--	--	--	--	--	--	--	--	--	--
	+6	16	--	--	--	--	--	--	--	--	--	--
	+9	16	--	--	--	--	--	--	--	--	--	--
100	14	80	--	8	7	7	--	13	12	9	44	--
	+2	16	--	4	--	--	--	--	--	--	--	10
	+4	16	8	--	--	--	--	--	--	--	--	--
	+6	16	--	4	--	--	--	--	--	--	--	14
	+9	16	--	--	--	--	--	--	--	--	--	4
2500	14	76	--	6	24	4	--	2	3	7	41	--
	+1	14	--	--	--	--	--	--	--	--	--	8
	+2	16	--	--	--	--	--	--	--	--	--	10
	+4	16	--	1	--	--	--	--	--	--	--	8
	+9	14	--	--	--	--	--	--	--	--	--	10

+/- minimal, + slight, ++ moderate, +++ marked

Plasma tyrosine concentrations

Time point		Dose level (ppm)				
		0[a]	5	100	0[a]	2500
Treatment	24 hours	---	---	---	126	2197**
	Week 1	130	1190**	2022**	---	---
	Week 14	155	1283**	2142**	192	1995*
Recovery	+1 week	105	128**	152**	120	408**
	+2 weeks	155	156	164	113	423**
	+4 weeks	107	124*	130**	111	146
	+9 weeks	181	193	191	111	134**

[a] respective control group

Biochemical investigations

Parameter	Time point (week)		Dose Group (ppm)				
			0	5	100	0	2500
TAT[1]	Treatment	14	3.391	3.891	4.067	2.352	3.913**
	Recovery	+1	---	---	---	2.279	2.908
		+2	3.080	3.044	2.375	---	---
		+4	2.599	2.705	2.667	---	---
		+6	2.985	3.022	2.868	---	---
		+9	2.351	2.634	2.540	2.003	1.968
HPPD[2]	Treatment	14	0.880	0.099**	0.031**	0.141	0.005**
	Recovery	+1	---	---	---	0.291	0.052**
		+2	0.794	0.411**	0.353**	---	---
		+4	0.900	0.496**	0.560**	0.563	0.267**
		+6	0.858	0.521**	0.475**	---	---
		+9	1.030	0.664**	0.690**	0.207	0.161

	Time Point (week)		**Kidney**			**Liver**		
			0	5	100	0	5	100
Tyrosine (µM)	Treatment	14	387	1341**	1944**	143	1036**	1716**
	Recovery	+2	441	471	472	136	134	179
		+4	199	187	199	124	130	117
		+6	277	227	237	98	1377	141
		+9	206	211	233	129	120	126

[1]nmol HPPA/min/mg protein, [2]µl oxygen/min/mg protein

90 day dose response study (male rats) Non-guideline GLP compliant Alpk:AP_fSD rats, males only (16/dose) Mesotrione (96.8% purity) Anonymous (1997d) RAR B.6.8.2.2	0, 0.5, 1, 3, 4, 5, 7.5, 10 or 100 ppm Equivalent to 0, 0.04, 0.09, 0.27, 0.35, 0.44, 0.67, 0.89 and 9.0 mg/kg bw/d	For ocular findings, see table below. Other significant findings (by dose) No significant findings at <5ppm. **5 ppm (0.44 mg/kg bw/d)** ↑ liver weight (9.8%) **7.5 ppm (0.67 mg/kg bw/d)** ↑ liver weight (12.0%) ↑ renal tubular basophilia (7/16 animals, cf. 5/16 controls) **10 ppm (0.89 mg/kg bw/d)** ↑ renal tubular basophilia (9/16 animals, cf. 5/16 controls) ↑ liver weight (11.5%) **100 ppm (9.0 mg/kg bw/d)** ↑ renal tubular basophilia (8/16 animals, cf. 5/16 controls) ↑ liver weight (14.8%) ↓ hepatic glycogen (2/16 animals, cf. 0/16 in controls) Ocular findings:

Finding	**Dose group (ppm)**								
	0	0.5	1	3	4	5	7.5	10	100
Cloudy eyes	0	0	0	0	0	1	1	3	13
Hazy/slight	1	0	0	0	0	1	2	2	3
Hazy/moderate	0	0	0	0	0	0	0	0	7
Opaque/slight	0	0	0	0	0	0	2	4	4
Opaque/moderate	0	0	0	0	0	0	1	0	8
Vascularisation	0	0	0	0	0	0	1	2	16

Plasma tyrosine & urine analyses

	Plasma tyrosine concentration (µM)								
	0	**0.5**	**1**	**3**	**4**	**5**	**7.5**	**10**	**100**
24hr	110	114	153**	1342**	1525**	2130**	2307**	2378**	3023**
Wk 1	125	490**	523**	937**	1219**	1463**	1952**	2723**	3495**
Wk 14	113	228**	431**	915**	1241**	1482**	1934**	1771**	2772**
	Urine phenolic acids (mg eq)								
Conju-gated	0.53	1.13	0.81	---	0.81	1.10	1.31	2.31	3.03
Free	0.47	1.08	1.65	2.14	4.81	2.38	1.52	3.81	10.44
Total	1.00	2.21	2.46	2.14	5.62	3.48	2.83	6.12	13.47

Hepatic enzyme activity

	Enzyme activity								
	0	**0.5**	**1**	**3**	**4**	**5**	**7.5**	**10**	**100**
TAT[1]	1.724	1.890	2.154	2.610**	2.568**	2.551**	2.779**	2.414**	2.325**
HPPD[2]	0.268	0.085**	0.049**	0.055**	0.048**	0.020**	0.026**	0.019**	0.008**

[1]nmol HPPA/min/mg protein

[2]µl oxygen/min/mg protein

90 day dose response study (female rats) Non-guideline GLP compliant Alpk:AP$_f$SD rats, females only (12/dose) Mesotrione (96.8% purity) Anonymous (1997e) RAR B.6.8.2.3	0, 1, 5, 10, 50, 100, 1000 or 2500 ppm Equivalent to 0, 0.09, 0.48, 0.95, 4.8, 9.5, 95 or 237 mg/kg bw/d	Ocular findings: **Finding** / **Dose group (ppm)**: 0 / 1 / 5 / 10 / 50 / 100 / 1000 / 2500 Cloudy eyes: 0 / 0 / 0 / 0 / 0 / 0 / 7 / 7 **Corneal Opacity** (number of eyes) Hazy/slight: 0 / 0 / 0 / 0 / 0 / 2 / 10 / 11 Hazy/moderate: 0 / 0 / 0 / 0 / 0 / 0 / 4 / 0 Opaque/slight: 0 / 0 / 0 / 0 / 0 / 0 / 1 / 1 Opaque/moderate: 0 / 0 / 0 / 0 / 0 / 0 / 1 / 3 Vascularisation: 0 / 0 / 0 / 0 / 0 / 0 / 4 / 3 Plasma tyrosine & urine analyses **Plasma tyrosine concentration (µM)** **0 / 1 / 5 / 10 / 50 / 100 / 1000 / 2500** Week 2: 112 / 128 / 217** / 440** / 843** / 1187** / 1556** / 1668** Week 5: 123 / 144 / 218** / 322** / 731** / 1021** / 1431** / 1504** Week 14: 127 / 147 / 219** / 249** / 620** / 836** / 1593** / 1534** **Urine phenolic acids (mg eq)** Conjugated: nd / nd / nd / nd / nd / 1.82 / 1.28 / 2.78 Free: nq / nq / nq / nq / nq / nq / 5.67 / 20.22 Total: nd / nd / nd / nd / nd / 1.82 / 6.95 / 23.00 Hepatic enzyme activity Enzyme / Wk / **Enzyme activity** 0 / 1 / 5 / 10 / 50 / 100 / 1000 / 2500 TAT[1] / 2: 1.503 / 1.996 / 2.479** / 2.749** / 3.015** / 2.964** / 3.507** / 3.805** / 5: 1.734 / 1.725 / 2.069 / 1.866 / 2.085 / 2.107 / 3.963** / 3.248** / 14: 3.390 / 3.044 / 3.752 / 3.785 / 4.126 / 3.787 / 4.317* / 4.855** HPPD[2] / 2: 1.782 / 0.789** / 0.221** / 0.203** / 0.205** / 0.120** / 0.028** / 0.017** / 5: 1.274 / 0.366** / 0.153** / 0.112** / 0.105** / 0.069** / 0.041** / 0.030** / 14: 2.373 / 0.945** / 0.321** / 0.326** / 0.246** / 0.171** / 0.057** / 0.019** [1]nmol HPPA/min/mg protein [2]µl oxygen/min/mg protein

<table>
<tr>
<td>90 day study in rats to investigate ocular end points

None-guideline

GLP status unknown

Alpk:AP$_f$SD rats, males only (12/dose)

Mesotrione (95.1% purity)

Anonymous (1995a)

RAR B.6.8.2.4</td>
<td>0, 10, 20, 50 or 125 ppm

Equivalent to 0, 0.9, 1.7, 4.3 and 10.7 mg/kg bw/d</td>
<td>Ocular findings
<table>
<tr><th></th><th colspan="5">Dose level (ppm)</th></tr>
<tr><td></td><td>0</td><td>10</td><td>20</td><td>50</td><td>125</td></tr>
<tr><td colspan="6">Clinical observation</td></tr>
<tr><td>Eye opaque[1]</td><td>0</td><td>3</td><td>9</td><td>8</td><td>9</td></tr>
<tr><td colspan="6">Opthalmoscopy</td></tr>
<tr><td>Hazy/slight[2]</td><td>1</td><td>1</td><td>0</td><td>2</td><td>0</td></tr>
<tr><td>Hazy/moderate[2]</td><td>0</td><td>2</td><td>4</td><td>2</td><td>4</td></tr>
<tr><td>Hazy/marked[2]</td><td>0</td><td>1</td><td>6</td><td>4</td><td>10</td></tr>
<tr><td>Opaque/slight[2]</td><td>0</td><td>3</td><td>1</td><td>1</td><td>2</td></tr>
<tr><td>Opaque/moderate[2]</td><td>0</td><td>0</td><td>1</td><td>0</td><td>0</td></tr>
<tr><td>Opaque/marked[2]</td><td>0</td><td>0</td><td>0</td><td>0</td><td>2</td></tr>
<tr><td>Vascularisation[2]</td><td>0</td><td>2</td><td>8</td><td>8</td><td>14</td></tr>
<tr><td>Ghost vascularisation[2]</td><td>0</td><td>1</td><td>0</td><td>1</td><td>0</td></tr>
</table>
[1]Number of animals
[2]Number of eyes

Other effects:
↑ liver weight at ≥ 10 ppm (0.9 mg/kg bw/d): 11.5 – 14.3% (no clear dose response)</td>
</tr>
</table>

Ocular toxicity development and reversibility study in rats Non-guideline GLP compliant Alpk:APfSD rats, males only (16 or 40/dose) Mesotrione (96.8% purity) in the diet Anonymous (1997c) RAR B.6.8.2.5	0, 2500 ppm Equivalent to 0 and 272 mg/kg bw/d 16 animals received 0 ppm and 40 animals received 2500 ppm. After 5 weeks, half of the control and half of the treated animals were allocated to a reversibility phase and were fed control diet for 8 weeks.	**Ocular effects (clinical observations)** After 5 weeks, 22/40 animals at 2500 ppm had cloudy eyes (cf. 0/16 controls) **Ophthalmoscopy findings** **Plasma tyrosine concentrations** **Microscopic findings**

Observation	**Treatment phase**					**Reversibility phase**					
Week	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**10**		**12**	
Group (ppm)	**2500**					**2500**		**0**	**2500**	**0**	**2500**
Opaque/slight	2	14	23	29	23	10	1	-	-	-	-
Opaque/moderate	-	-	1	4	12	1	-	-	-	-	-
Opaque/marked	-	-	-	1	4	-	-	-	-	-	-
Hazy/slight	-	-	-	-	3	2	-	1	1	2	1
Hazy/moderate	-	-	-	-	2	3	-	-	-	-	-
Hazy/marked	-	-	-	-	-	2	1	-	-	-	-
Vascularisation (ghost)	- -	- -	- -	3 -	7 -	9 -	6 9	- -	1 11	-	- 11

Dose Group (ppm)	**Plasma tyrosine concentration (µM)**				
	Treatment phase		**Reversibility phase**		
	Week 1	**Week 2**	**Week 6**	**Week 7**	**Week 14**
0	422	143	167	127	97
2500	222	2956**	2536**	1719**	113**

Finding	**Week 5**		**Week 14**	
	0	2500	0	2500
Number of animals	8	25	8	15
Corneal keratitis	0	11	---	---
Polymorphonuclear leucocyte infiltration	0	10	---	---
Corneal vessels	---	---	0	8
Corneal stromal fibroblasts	---	---	0	8
Corneal epithelial disruption			0	3
Harderian gland mononuclear infiltration	1	2	0	2

2 year dietary toxicity and oncogenicity study in rats OECD 453 GLP compliant Alpk:AP_fSD rats (64/sex/dose level) Mesotrione (96.8% purity) in the diet Anonymous (1997f) RAR B.6.5.1.1	0, 1, 2.5, 7.5, 100 and 2500 ppm Equivalent to: 0.0, 0.06, 0.16, 0.48, 6.5 and 160 mg/kg bw/d in males 0.0, 0.09, 0.19, 0.57, 7.7 and 190 mg/kg bw/d in females	***For neoplastic effects, see Section 4.10*** ***Non-neoplastic effects by dose:*** **1 ppm (0.06/0.09 mg/kg bw/d)** No treatment-related effects **2.5 ppm (0.16 / 0.19 mg/kg bw/d)** *Ophthalmoscopy* ↑ corneal opacity (males) *Kidney* ↑ incidence of enlarged kidney (males 50%, cf. 23% of controls), roughened kidney (males 65%, cf. 40% of controls), renal cysts (males 35%, cf. 29% of controls), pale kidney (males 55%, cf. 50% of controls) *Liver* ↑ incidence of pale liver (males 40%, cf. 25% of controls), enlarged liver (females 5%, cf. 0% of controls) **7.5 ppm (0.48 / 0.57 mg/kg bw/d)** *Body weight* ↓ terminal body weight (males, 12.3%) *Clinical chemistry* ↓ ALT (males, 15.1 – 62.6%), ↓ AST (males, 14.0 – 27.6%) *Eyes* ↑ corneal opacity (males) *Kidneys* ↑ weight at interim kill (males, 14.3%) ↑ renal pelvis dilatation at interim kill (males, 4/12, cf. 1/11 in controls) ↑ incidence of enlarged kidney (males 42%, cf. 23% of controls), roughened kidney (males 63%, cf. 40% of controls), renal cysts (males 50%, cf. 29% of controls), pale kidney (males 62%, cf. 50% of controls), ↑ chronic progressive glomerulonephropathy (males) *Liver* ↑ weight (males, interim kill: 17.5%) ↑ incidence of pale liver (males 60%, cf. 25% of controls), enlarged liver (males 10%, females 5% cf. 2% and 0% of controls), proliferate cholangitis (12/12 males, 8/11 females, cf. 7/11 and 7/12 controls) *Adrenals* ↓ weight at terminal kill (males, 18.9%) ↑ pale adrenals (males 10%, cf. 2% in controls) *Thyroid* ↑ follicular cysts with hyperplastic epithelium (males, 5 cf. 1 in controls) *Seminal vesicles* ↑ reduced seminal vesicles (23%, cf. 13% of controls) *Sciatic nerve* ↑ severity of demyelination (males only)

		100 ppm (6.5 / 7.7 mg/kg bw/d) *Body weight* ↓ terminal body weight (males, 11.4%) *Clinical chemistry* ↑ urea (females, 13.5%), ↓ ALT (males, 13 – 59.4%), ↓ AST (males, 10.4 - 30.4%), ↑ urine specific gravity (both sexes), ↓ urine pH (males: 6.31, cf. 6.62 in controls) *Eyes* ↑ corneal opacity (both sexes) ↑ harderian gland mononuclear infiltration (males, 10/64 cf. 2/64 controls) ↑ harderian gland porphyrin deposition (males, 34/64, cf. 25/64 controls) *Kidneys* ↑ weight at interim kill (males, 13.3%) ↑ renal pelvis dilatation at interim kill (males, 2/12, cf. 1/11 in controls) ↑ incidence of enlarged kidney (males 25%, cf. 23% of controls), roughened kidney (males 63%, cf. 40% of controls), renal cysts (males 35%, cf. 29% of controls), pale kidney (females 12%, cf. 2% of controls), ↑ chronic progressive glomerulonephropathy (males) *Liver* ↑ weight (males: interim kill 15.2%, terminal kill 13.8%. Females: interim kill 11.0%, terminal kill 10.7%) ↑ incidence of pale liver (males 52%, cf. 25% of controls), enlarged liver (females 8%, cf. 0% of controls), accentuated lobes (females 10%, cf. 2% of controls), proliferate cholangitis (12/12 males, 10/11 females at interim kill, cf. 7/11 and 7/12 controls), ↑ hepatocyte fat vacuolation (39/64 males, 16/34 females, cf. 17/64 and 8/64 controls) *Thyroid* ↑ follicular cysts (males, 3 cf. 1 in controls), follicular cysts with hyperplastic epithelium (males, 7 cf. 1 in controls) and squamous cysts (females, 2 cf. 0 in controls). *Sciatic nerve* ↑ severity of demyelination (both sexes) *Other effects* ↑ testes weight at terminal kill (29.4%) ↑ reduced seminal vesicles (42%, cf. 13% of controls) ↑ focal swelling of the uterus (12%, cf. 4% of controls) **2500 ppm (160 / 190 mg/kg bw/d)** *Body weight* ↓ terminal body weight (males, 14.4%) *Haematology* ↑ eosinophil (females from week 53, 34.1 – 142%), ↓ platelet count (males 4.1 – 16.1%, females, 7.5 – 17.5%), ↓ APTT (males, 21.6) *Clinical chemistry* ↑ urea (females, 14.9%), ↓ AP (males, 15.9 – 21.2%), ↓ ALT (males, 13%), ↓ AST (males, 9.8%) ↓ urine volume (males 21.4%, females 6.6%), ↑ urine specific gravity, ↓ urine pH (males: 6.08, cf. 6.62 in controls) *Eyes* ↑ corneal opacity (both sexes) ↑ Harderian gland mononuclear infiltration (males) ↑ Harderian gland porphyrin deposition (males)

		Kidneys ↑ weight at interim kill (males 21.6%, females 9.2%) ↑ renal pelvis dilation at interim kill (males, 4/12, cf. 1/11 in controls; females, 1/12, cf. 0/12 controls) ↑ incidence of enlarged kidney (males 35%, cf. 23% of controls), roughened kidney (males 58%, cf. 40% of controls), renal cysts (males 48%, cf. 29% of controls), pale kidney (males 65%, cf. 50% of controls; females 10%, cf. 2% of controls), ↑ chronic progressive glomerulonephropathy (males) *Liver* ↑ weight (males: interim kill 18.4%, terminal kill 19.8%. Females: interim kill: 13.6%, terminal kill: 7.5%) ↑ incidence of pale liver (males 54%, cf. 25% of controls; females 27%, cf. 6% of controls), enlarged liver (males 8%, females 6%, cf. 2% and 0% of controls), accentuated lobes (males 8%, females 10%, cf. 4% and 2% of controls), proliferate cholangitis (9/11 males, 12/12 females, cf. 7/11 and 7/12 controls), hepatocyte fat vacuolation. *Thyroid* ↑ squamous cysts (females, 5 cf. 0 in controls) ↑ follicular cysts (males, 5 cf. 1 in controls) ↑ follicular cysts with hyperplastic epithelium (males, 5 cf. 1 in controls) *Sciatic nerve* ↑ severity of demyelination (both sexes) *Other effects:* ↑ testes weight at terminal kill (20.0%) ↑ reduced seminal vesicles (35%, cf. 13% of controls) ↑ focal swelling of the uterus (12%, cf. 4% of controls)

1. *Guideline 28 day study in Alpk:AP$_f$SD rats*

Each animal (6/sex/group) was administered mesotrione in the diet at 0, 131, 656, 1315, 1871 and 2464 mg/kg bw/day in males and 0, 133, 651, 1324, 1916 and 2424 mg/kg bw in females.

Food consumption was reduced in males at 656 mg/kg bw/d and females at 1916 mg/kg bw/d. Bodyweight gain over the study period was reduced in all treated groups. Significant reductions in mean body weight (>10%) were observed from ≥1315 mg/kg bw/d in males and ≥1916 mg/kg bw/d in females. Plasma creatinine levels were significantly increased in females at ≥651 mg/kg bw/d ppm and in males at ≥1315 mg/kg bw/d ppm, which is consistent with the kidney being the target organ (see below).

The target organ was the kidney in both sexes. Increased tubular hyaline droplet formation was observed in all treated males and the majority of treated females. Transitional epithelial hyperplasia of the renal pelvis was observed at low incidence in all groups of treated males (but without a clear dose-response relationship) and in females at 1324 mg/kg bw/d. Renal pelvis dilatation and hydronephrosis were increased in some treated groups, however no clear dose-response relationship was observed. Urinalysis results were consistent with the kidney being a target organ. Urine specific gravity was increased in all treated groups (significantly in females at133 mg/kg bw/d). Blood was detected in the urine of males at ≥656 mg/kg bw/d (qualitatively) and quantitatively in all treated groups. Renal epithelial cells were also detected in the urine of treated males with increased frequency.

Findings consistent with adaptive change were noted in the liver of both sexes, i.e., increased

relative weight (up to 9.3% in males and 15.2% in females, no clear dose-response relationship) and increased APDM activity from 1315/1324 mg/kg bw/d.

2. Guideline 90 day study in Alpk:AP$_f$SD rats

Rats (12/sex/dose) were administered mesotrione in the diet at 0, 1, 125, 1250 or 12500 ppm for 90 days (equivalent to 0, 0.09, 11.0, 112, 1111 mg/kg bw/d in males and 0.1, 12.8, 126 and 1213 mg/kg bw/d in females).

Weight gain was decreased in all groups of treated males and in females at ≥11 mg/kg bw/d. Mean food consumption was significantly lower in both sexes intermittently at <12.8 mg/kg bw/d, and mean overall food utilisation was significantly lower in males at ≥11 mg/kg bw/d and in females at 1213 mg/kg bw/d. Significant (>10%) reductions in mean terminal body weights were seen in males at the top dose only.

Urine pH levels were slightly (but statistically significantly) lower in both sexes at 1111/1213 mg/kg bw/d and in males at 112 mg/kg bw/d, which may be attributable to the acidic nature of the test material. Small quantities of renal epithelial cells were detected in the urine of a number of males at ≥112 mg/kg bw/d ppm and in all groups of treated females, however there was no clear dose response relationship.

The target organs were the eyes and kidneys. 'Eye opaque' was noted in males at ≥ 11 mg/kg bw/d and females at ≥ 126 mg/kg bw/d. Ophthalmoscopy revealed increased incidence and severity of corneal opacity in males and females at ≥ 11 mg/kg bw/d . Males were affected to a greater extent than females. Corneal vascularisation was also noted in males at ≥ 11 mg/kg bw/d ppm and females at ≥ 126 mg/kg bw/d. Microscopic examination revealed unilateral or bilateral corneal keratitis in both sexes at ≥11 mg/kg bw/d. Again, males were affected to a greater degree than females. Keratitis was characterised by inflammatory cell infiltration of the stroma and epithelium with focal disruption/disorganisation of the corneal epithelium, and was often associated with corneal vascularisation.

Effects in the kidneys were seen in males only and consisted of increased relative weight at ≥ 11 mg/kg bw/d (up to 13.8%) and increased incidences of renal tubular hyaline droplet formation at 11 and 1111 mg/kg bw/d. No clear dose response was seen for either effect.

Relative liver weights were significantly increased in both sexes at ≥ 11 mg/kg bw/d, which, in the absence of histopathology findings is considered to be an adaptive change.

3. Guideline 90 day study in Alpk:AP$_f$SD rats

In the second guideline 90 day study, each animal (12/sex/dose) was administered mesotrione in the diet at 0, 0.23, 0.47, 0.71 and 14.5 mg/kg bw/d in males and 0, 0.21, 0.41, 0.63 and 12.5 mg/kg bw/d in females.

Urinalysis revealed slightly (but significantly) increased specific gravity and decreased pH in males at 14.5 mg/kg bw/d. Urinary ketones and protein were noted in treated animals with increased frequency.

The target organs were the eyes (in both sexes) and the kidneys (males only). Ocular opacity was noted in males from 0.71 mg/kg bw/day and in females from 12.5 mg/kg bw/d. Ophthalmoscopy revealed dose-related increases in the incidence and severity of corneal opacity and in the incidence of corneal vascularisation in males (≥ 0.47 mg/kg bw/d). Corneal findings were noted in females at the top dose only. Microscopic examination revealed slight or moderate keratitis in 4/12 males at 0.71 mg/kg bw/d and 7/12 males at 14.5 mg/kg bw/d. In addition, one male had minimal corneal epithelial disorganization. In females, slight keratitis was noted in one female at 12.5 mg/kg bw/d.

Mean absolute kidney weights were increased in males at 0.71 and 14.5 mg/kg bw/d, however no significant effects were seen on relative weights or weights corrected for terminal bodyweight. Renal pelvic dilatation was slightly increased in treated males, however there was no clear dose-response relationship (incidences were 0/12, 1/12, 2/12, 1/12 and 1/12 at 0, 0.23, 0.47, 0.71 and 14.5 mg/kg bw/d respectively). Chronic progressive glomerulonephropathy and hydronephrosis were increased in males in the top dose group (tissues at other doses were not examined microscopically).

Liver weights (absolute and weights corrected for terminal bodyweight) were significantly increased in all treated groups of males (up to 17.7%). Liver weights were also significantly increased in females at 12.5 mg/kg bw/d. As no histopathological findings are reported, the increase in weight is likely to be an adaptive response.

4. Non-guideline 90 day reversibility studies in Alpk:AP$_f$SF rats

Male rats (40/dose level) were fed diets containing mesotrione at 0, 0.37, 7.5 and 192 mg/kg bw/d for 90 days (Weeks 1-14) followed by recovery periods of 0, 1, 2, 4, 6 or 9 weeks. Ophthalmoscopy was performed at the end of the 90 day treatment period and prior to termination. Blood samples were taken for analysis of plasma tyrosine levels at various points during the study period. Liver samples (4/dose level/time point) were analysed for TAT and HPPD activity. Liver and kidney samples were also analysed for tyrosine concentration.

Treated animals showed an increased incidence of 'cloudy eyes'. Ophthalmoscopy revealed dose related increases in the incidence and severity of corneal opacity and the incidence of corneal vascularisation at 90 days. The incidence and degree of opacity decreased rapidly during the recovery period. Corneal vascularisation resolved to ghost vascularisation. Corneal opacity was not apparent in animals following a recovery period of 8 weeks.

HPPD activity was significantly decreased in all treated groups at the end of the treatment period. The degree of HPPD inhibition decreased during the recovery phase, however values in some treated groups were still significantly lower at termination.

TAT activity was increased in treated groups, significantly at 192 mg/kg bw/d. Activity was comparable in all groups at the end of the recovery phase.

Liver and kidney tyrosine levels were significantly increased at the end of the treatment period. Levels in both organs decreased rapidly during the recovery phase and were comparable to controls from Week 16. Plasma tyrosine concentrations were significantly increased at 192 mg/kg bw/d from 24 hours, and at 0.37 and 7.5 mg/kg bw/d animals from Week 1 (the earliest sample point for these groups). Levels decreased during the recovery period and were comparable to controls at Week 16 (0.37 and 7.5 mg/kg bw/d) and Week 23 (192 mg/kg bw/d).

In summary, mesotrione was found to produce a dose-related hypertyrosinaemia in male rats at ≥0.37 mg/kg bw/d. Hypertyrosinaemia correlated with HPPD inhibition and TAT induction.

5. *Non-guideline 90 day dose response study in male Alpk:AP$_f$SF rats*

Male rats (16/dose level) were fed diet containing mesotrione at 0, 0.04, 0.09, 0.27, 0.35, 0.44, 0.67, 0.89 and 9.0 mg/kg bw/d. An additional control group was included in this study. Ophthalmoscopy was performed pre-test and prior to termination. Blood samples were analysed for plasma tyrosine levels 24 hours and 1 week after the start of the study (4/dose level) and in all animals at termination. Liver samples (4/dose level) were analysed for TAT and HPPD activity.

The incidence of cloudy eyes was increased in animals at ≥0.35 mg/kg bw/d. Ophthalmoscopy revealed corneal vascularization, and increasing incidence and severity of corneal opacity at ≥0.67 mg/kg bw/d.

There was a dose-related decrease in HPPD activity in all treatment groups.

TAT activity was increased in all treatment groups (statistically significant at ≥ 0.27 mg/kg bw/d), however there was no clear dose-response.

There was a dose-dependent increase in plasma tyrosine levels, which were significantly increased at all doses/time points (except 0.04 mg/kg bw/d after 24 hours). The increase in plasma tyrosine is consistent with the reduction in HPPD activity. At Week 14, tyrosine concentrations were similar to or less than concentrations at Week 1. Increased conjugated and free phenolic acid were noted in the urine of all treated groups.

The incidence of renal tubule basophilia was slightly increased at ≥0.67 mg/kg bw/d compared to controls. Electron microscopy of kidney sections did not reveal any treatment-related findings. There was a dose-related increase in mean liver weights at ≥0.35 mg/kg bw/d (>10% at doses ≥0.67 mg/kg bw/d). Depleted hepatic glycogen was noted in two top dose animals.

In summary, mesotrione was found to cause a dose-related hypertyrosinaemia in all groups of treated rats (≥0.04 mg/kg bw/d). Correlation was seen between plasma concentrations of tyrosine, urinary excretion of phenolic acids, increased liver weights, HPPD inhibition and TAT induction.

6. *Non-guideline 90 day dose response study in female Alpk:AP$_f$SD rats*

Female rats (12/dose level) were fed diet containing mesotrione at 0, 0.09, 0.48, 0.95, 4.8, 9.5, 95 and 237 mg/kg bw/d. An additional 8 animals/dose level were sacrificed on Days 8 and 29. Ophthalmoscopy was performed prior to termination. Plasma tyrosine concentration were measured during Weeks 2 and 5 (4/dose level) and at termination (all animals). Urine samples (4/dose level) were collected during Weeks 2, 5 and 13 for analysis of ketone content. Liver samples (4/dose level) were analysed for TAT and HPPD activity.

Cloudy eyes were noted in animals at 95 and 237 mg/kg bw/d. Ophthalmoscopy revealed dose related increases in the incidence and severity of corneal opacity at ≥9.5 mg/kg bw/d. Opacity was accompanied by corneal vascularisation in some animals at ≥95 mg/kg bw/d.

There was a dose-related decrease in HPPD activity in all treated groups, and a dose-related increase in TAT activity.

A dose-related increase in plasma tyrosine concentrations was seen at ≥5 ppm at all time points. Concentrations at 95 and 237 mg/kg bw/d were comparable, and the degree of hypertyrosinaemia did not significantly change with time. Urinalysis revealed increased amounts of conjugated phenolic acids at ≥9.5 mg/kg bw/d and increased free phenolic acids at ≥95 mg/kg bw/d. Phenolic

acids were identified by NMR as 4-hydroxyphenylpyruvate (HPPA), 4-hydroxyphenyllactate (HPLA) and 4-hydroxyphenylacetate (HPAA).

In summary, a dose-related hypertyrosinaemia was observed in female rats administered mesotrione at ≥0.48 mg/kg bw/d. This correlated with inhibition of HPPD, increased TAT activity and the excretion of phenolic acid metabolites of tyrosine.

7. Non-guideline 90 day study looking at ocular effects in male Alpk:AP$_f$SD rats

Male rats (12/dose level) were administered mesotrione in the diet at 0, 0.9, 1.7, 4.3 and 10.7 mg/kg bw/d. Ophthalmoscopy was performed during the week prior to termination.

Ophthalmoscopy revealed dose-related increases in the incidence and severity of corneal opacity and in the incidence of corneal vascularisation.

8. Non-guideline ocular toxicity development and reversibility study in Alpk:AP$_f$SD rats

In a study investigating ocular toxicity, male rats were administered mesotrione at 0 ppm (16 animals) or 2500 ppm (40 animals) in the diet for 5 weeks (equivalent to 0 and 272 mg/kg bw/d). After 5 weeks, half of the control animals and approximately half of the treated animals (those showing ocular effects as assessed by ophthalmoscopy) were allocated to a reversibility phase and fed control diet for an additional eight weeks. The remaining animals were terminated.

The clinical observation of 'cloudy eyes' was noted in more than half of treated animals, but not in control animals. Corneal opacity in treated animals was apparent at Week 1, increasing in incidence and severity to Week 6. Corneal vascularisation was noted from Week 4, increasing in incidence to Week 6. The incidence and severity of corneal opacity decreased rapidly during the reversibility phase of the study and was comparable to controls at Week 10. Corneal vascularisation resolved to ghost vascularisation at Week 10, which was still apparent at Week 12. Microscopically, corneal keratitis was noted in treated animals terminated at Week 5. This finding was characterised by polymorphonuclear leucocytic infiltration of the outer corneal stroma and epithelium with or without corneal epithelial disruption/ disorganisation. Some animals with keratitis also showed epithelial hyperplasia and vascularisation.

After the reversibility phase, no evidence of corneal keratitis was seen. Minimal or slight disruption of the corneal epithelium was noted in three treated animals. Rats with ghost vascularisation showed histological evidence of remaining corneal vessels and the presence of fibroblasts in the sub-epithelial stroma.

Plasma tyrosine concentrations in treated animals were significantly higher from Weeks 2-7. Levels decreased during the reversibility phase and were comparable to controls at termination.

In summary, a high incidence of corneal lesions was seen in rats fed 272 mg/kg bw/d mesotrione, with effects apparent after one week of treatment. Marked increases in plasma tyrosine concentrations were also seen in treated animals. Plasma tyrosine levels in treated animals were comparable to controls following a recovery period of 8 weeks. Corneal opacity resolved during the recovery period and vascularisation resolved to ghost vascularisation. Histological evidence of healing was also observed.

9. Guideline 2 year carcinogenicity study in Alpk:APfSD rats

Only non-neoplastic effects are discussed here; neoplastic effects are discussed in Section 4.10 Carcinogenicity.

Rats (64/sex/dose level) were administered mesotrione in the diet at 0.0, 0.06, 0.16, 0.48, 6.5 and 160 mg/kg bw/d (males) or 0.0, 0.09, 0.19, 0.57, 7.7 and 190 mg/kg bw/d (females) for up to 104 weeks. 12 animals/sex/dose were terminated at 52 weeks. Two additional groups (20/sex) were administered mesotrione at 1.0 or 2.5 ppm for the investigation of ocular toxicity. Ophthalmoscopy was performed pre-test, during Weeks 26, 52, 78 and prior to termination.

Weight gain was decreased in males at all dose levels and slightly decreased in females from ≥0.19 mg/kg bw/d. Food consumption was significantly lower at 160/190 mg/kg bw/d in males and females respectively. Terminal body weight was statistically significantly lower than controls in males at ≥ 0.48 mg/kg bw (↓14.4% at the top dose).

The target organs were the eyes and kidney. Cloudy eyes were observed with increased incidence in all groups of treated males and in females at ≥0.48/0.57 mg/kg bw/d, the time of onset decreasing with increasing dose. Ophthalmoscopy revealed an increased incidence of corneal opacity (as a result of keratitis) in males at ≥0.16 mg/kg bw/d and in females at ≥7.7 mg/kg bw/d ppm. The severity of opacity was dose related and was apparent from 26 weeks (the earliest examination time) in both sexes. Opacity was frequently accompanied by corneal vascularisation, particularly at higher dose levels. The severity of Harderian gland porphyrin deposition was increased in males at ≥0.48 mg/kg bw/d. In males, mononuclear cell infiltration of the Harderian gland were slightly increased, and the severity of Harderian gland porphyrin deposition was increased at ≥0.48 mg/kg bw/d.

At the interim kill, mean relative kidney weights were significantly increased in males at ≥0.48 mg/kg bw/d and in top dose females. The incidence of renal pelvis dilatation was increased in males at ≥0.48 mg/kg bw/d. The incidences of enlarged, roughened, cystic or pale kidneys were slightly increased in males at ≥0.16 mg/kg bw/d, however no clear dose response relationships were seen. The incidence of chronic progressive glomerulonephropathy was comparable in all groups of males, however the severity of this finding was increased in males at ≥ 0.48 mg/kg bw/d. Treatment related renal change in females was limited to an increased incidence of pale kidneys at ≥ 7.7 mg/kg bw/d. Plasma urea concentrations were slightly (but significantly) increased at ≥ 7.7 mg/kg bw/d in females at the interim kill. Decreased urine volume, increased specific gravity and decreased pH (attributable to the acidic nature of the test material) were noted in both sexes at ≥6.5/7.7 mg/kg bw/d at 13 weeks. Ketones in the urine and haematuria were detected with increased incidence in both sexes at ≥6.5/7.7 mg/kg bw/d.

Mean relative liver weights were significantly increased in males at ≥0.48 mg/kg bw/d (19.8% at the top dose) and in females at ≥7.7 mg/kg bw/d (up to 13.6%). Liver enlargement (≥0.19 mg/kg bw/d) and accentuation of the hepatic lobes (≥7.7 mg/kg bw/d) were slightly increased in females. At the interim kill, the incidence of liver proliferative cholangitis was slightly increased in treated animals of both sexes. Hepatocyte fat vacuolation was seen with increased incidence in males at ≥0.48 mg/kg bw/d and in females at ≥ 7.7 mg/kg bw/d. The effects in the liver are difficult to interpret, as although there were some findings in treated animals, these generally occurred without a dose-response and the majority of findings were also present in controls to some extent. Overall, the effects in the liver are not considered to represent a significant or severe effect of dosing with mesotrione.

The incidence of cystic/hyperplastic thyroid was increased in males at ≥0.48 mg/kg bw/d and in females at ≥7.7 mg/kg bw/d. No follicular hyperplasia was observed.

Summary of oral studies in rats

Following repeated exposure to mesotrione, the target organs in rats were the eyes and kidneys, with males being more sensitive to these effects than females.

Effects in the eyes were consistent across studies and consisted of ocular opacity as a result of corneal keratitis, epithelial disruption and associated vascularisation. The effects were seen at doses ≥ 0.71 mg/kg bw/d in males (guideline 90 day study) and at doses ≥ 95 mg/kg bw/d in females (non-guideline 90 day dose response study). Eye effects were also seen in males after just 1 week in a non-guideline study, at a higher dose level (272 mg/kg bw/d). The effects in the eyes were shown to be reversible upon cessation of treatment.

The effects in the kidneys are difficult to interpret, as there are inconsistencies in the effects seen across studies of different durations. However, overall, it is considered that certain findings (notably the renal pelvic dilatation and the chronic progressive glomerulonephropathy) do occur with some consistency. These findings were seen in several studies at doses below the relevant guidance values for classification and, despite the inconsistencies, cannot be dismissed.

The studies also show that rats develop significantly elevated levels of plasma tyrosine (hypertyrosinaemia) after repeated dosing with mesotrione, with a dose-related increase in the activity of the enzyme TAT, and a dose-related decrease in the activity of HPPD. Plasma tyrosine levels, TAT and HPPD activity return to normal upon cessation of treatment.

Additional studies in the rat

In addition to the studies summarised above, three non-guideline studies are available which further investigate the effects seen in rats following repeated exposure to mesotrione and/or tyrosine. These studies are summarised in Table 11 (below) and include;

1. 21-day study to investigate effects of dietary tyrosine on ocular effects – animals dosed with 0, 0.5, 1.0, 2.5 and 5.0% tyrosine in a low protein diet.
2. 28-day study to investigate ocular effects, plasma tyrosine concentration and enzyme (HPPD and TAT) activity – dynamic dosing (0 or variable dietary levels of mesotrione).
3. 28-day study with varying combinations of mesotrione and/or tyrosine, investigating ocular effects, plasma tyrosine concentration and enzyme (HPPD and TAT) activity.

Table 11: Summary table of additional studies relevant to the assessment of repeated dose toxicity in rats by the oral route.

↑/↓ = increased/decreased compared to control. Unless otherwise stated, effects were seen in both sexes.
***significantly different to control (p < 0.05), **p < 0.01, ***p < 0.001**

<table>
<tr><th>Method</th><th>Dose Levels</th><th>Significant Toxicological Findings</th></tr>
<tr>
<td>21 day study looking at the effects of dietary tyrosine on ocular lesions in rats

None guideline

GLP status not known

Alpk:AP1SD rats, male weanlings (8/dose)

L-tyrosine (assumed 100% purity)

Anonymous (1995b)

RAR B.6.8.2.7</td>
<td>0, 0.5, 1.0, 2.5 and 5.0% w/w tyrosine in a low protein diet</td>
<td>Ophthalmic observations:
No ocular lesions were noted in animals in the 0.5 or 1.0% groups. In the animals exposed to 2.5% tyrosine, 6 lesions were noted by day 6 (cf. 0 lesions in controls). These animals were then terminated. In the animals exposed to 5.0% tyrosine, 6 lesions were noted by day 4 (cf. 0 lesions in controls). These animals were then terminated.

Microscopic Ocular findings:

<table>
<tr><th colspan="2" rowspan="2">Finding</th><th colspan="5">Dietary Tyrosine Level (% w/w)</th></tr>
<tr><th>0</th><th>0.5</th><th>1.0</th><th>2.5</th><th>5.0</th></tr>
<tr><td rowspan="2">Corneal keratitis</td><td>minimal</td><td>0</td><td>0</td><td>0</td><td>4</td><td>1</td></tr>
<tr><td>slight</td><td>0</td><td>0</td><td>0</td><td>0</td><td>3</td></tr>
<tr><td colspan="2">Epithelial disorganisation</td><td>0</td><td>0</td><td>0</td><td>0</td><td>2</td></tr>
<tr><td colspan="2">Polymorphs at filtration angle</td><td>0</td><td>0</td><td>0</td><td>1</td><td>4</td></tr>
<tr><td colspan="2">Retinal rosette</td><td>1</td><td>0</td><td>1</td><td>2</td><td>0</td></tr>
<tr><td colspan="2">Increased porphyrin Harderian gland</td><td>0</td><td>0</td><td>2</td><td>1</td><td>1</td></tr>
</table>
</td>
</tr>
</table>

28 day dynamic (i.e. variable dose) study in rats

None guideline

GLP compliant

Alpk:AP_fSD rats, male (20/dose)

Mesotrione (97.6% purity)

Anonymous (2000a)

RAR B.6.8.2.9

0 or variable dietary levels (100 to 0.3 ppm)

Two satellite groups (1 control, 1 variable dose treatment group) were included for measurement of plasma tyrosine and liver biochemistry

Overall mean dose: 2.39 mg/kg bw/d (range 0.025-13.03 mg mesotrione/kg/day)

Ocular effects

No treatment-related ocular effects were identified during the study

Other effects

↓ body weights in exposed animals (within 4% of the controls throughout the study)

↓ food consumption in exposed animals (within 6% of the controls throughout the study)

Plasma tyrosine levels

Group	Day	Last dose mesotrione prior to sampling		Mean mesotrione (nmol/ml)	N	SD
		ppm	mg/kg bw/d			
1	29	0	0	99.0	20	11.5
2	29	0.3	0.025	177.8	20	45.8
3	3	0	0	114.2	4	4.7
	15	0	0	110.3	4	6.5
4	3	100	12.59	1944.6	4	416.8
	5	30	3.68	1754.6	4	207.4
	7	10	1.21	1515.1	4	519.8
	9	3	0.37	825.3	4	339.4
	11	1	0.12	360.9	4	96.3
	15	0.3	0.03	164.5	4	15.3
	17	100	10.41	1929.6	4	178.7
	19	30	3.14	1580.0	4	80.8
	21	10	0.95	1419.0	4	164.0
	23	3	0.28	585.7	4	161.8
	25	1	0.09	301.4	4	41.9

Tyrosine aminotransferase and HPPD activity

Group	Day	Last dose mesotrione prior to sampling		HPPD activity Mean ul O_2/min/mg protein	TAT activity nmol HPPA/min/mg protein
		ppm	mg/kg bw/d		
1	29	0	0	0.912	1.991
2	29	0.3	0.025	0.131	2.001
3	3	0	0	1.190	1.078
	15	0	0	1.205	1.323
4	3	100	12.59	0.024	1.893
	5	30	3.68	0.037	2.256
	7	10	1.21	0.055	2.224
	9	3	0.37	0.090	1.860
	11	1	0.12	0.080	1.763
	15	0.3	0.03	0.218	1.551
	17	100	10.41	0.017	2.174
	19	30	3.14	0.041	2.252
	21	10	0.95	0.058	1.675
	23	3	0.28	0.066	1.594
	25	1	0.09	0.070	1.316

28 day oral study Non-guideline GLP compliant Alpk:AP_f SD rats, females only (8/group) Mesotrione (96.8% purity) and/or tyrosine in the diet Anonymous (1997g) RAR B.6.8.2.1	Group number and dose of mesotrione and/or tyrosine: 1. 0ppm / 0% 2. 100ppm / 0% 3. 100ppm / 0.5% 4. 100ppm / 1.0% 5. 100ppm / 2.5% 6. 0ppm / 0.5% 7. 0ppm / 1.0% 8. 0ppm / 2.5%	Ocular findings **Observation** / **Group**: **1** 0 / **2** 0 / **3** 8 / **4** 8 / **5** 8 / **6** 0 / **7** 0 / **8** 0 Corneal opacity[1] (minimal/slight): 0 / 1 / 8 / 1 / 0 / 0 / 0 / 0 Corneal opacity[1] (moderate/marked): 0 / 0 / 8 / 15 / 16 / 0 / 0 / 0 Vascularisation: 0 / 0 / 0 / 10 / 16 / 0 / 0 / 0 [1]number of eyes Plasma tyrosine concentrations & hepatic enzyme activity **Time point** / **Group Number**: **1** / **2** / **3** / **4** / **5** / **6** / **7** / **8** Plasma tyrosine concentration 24 hours: 112.5 / 1503 / 2129 / 2579 / 3517 / 136.4 / 142.5 / 268.9 Week 1: 141.5 / 1243 / 1733 / 2291 / 3729 / 160.5 / 183.7 / 244.6 Termination: 106.9 / 1189 / 1519 / 2803 / 2576 / 129.5 / 139.3 / 187.6 Hepatic enzyme activity at termination TAT1: 1.23 / 4.06** / 4.36*** / 5.71*** / 3.54*** / 1.13 / 1.52 / 2.74** HPPD2: 1.12 / 0.08** / 0.12** / 0.24** / 0.25** / 0.96 / 1.07 / 0.52* Other findings: Body weight Group 5: ↓ body weight (11.3%) Kidneys ↑ relative weight in animals administered mesotrione + tyrosine (groups 3-5, 7.7 – 15.5%) Liver ↑ relative weight in animals administered mesotrione (groups 2-5, 10.9, 11.2, 9.6 and 11%) ↑ relative weight in group 6 animals (6.1%)

1. *Non-guideline 21 day study into the effects of dietary tyrosine on ocular lesions in Alpk:AP$_f$SD rats*

Male weanling rats (8/dose group) were fed tyrosine (0.5, 1.0, 2.5 or 5.0% w/w) in a low protein diet for up to 21 days. Ophthalmoscopy was performed on Days 2-8, 11, 12, 14, 18 and 21 on all surviving rats. The eyes and Harderian glands of all animals were examined microscopically at termination.

Ophthalmoscopy revealed a rapid induction of ocular lesions in rats fed 2.5% (by Day 4) or 5.0% tyrosine (by Day 3). Lesions were initially seen as single or multiple focal areas of corneal opacity, which increased in size and merged to produce larger opaque areas

Ocular lesions were characterised microscopically by minimal to slight corneal keratitis, polymorphonuclear leukocyte infiltration of the outer corneal stroma and focal epithelial disorganisation. A small number of animals showed evidence of iritis with accumulation of polymorphonuclear leukocytes at the filtration angle.

In summary, rats administered high concentrations of tyrosine in a low protein diet were shown to rapidly develop ocular lesions, ophthalmoscopically and microscopically similar to those seen in rats administered mesotrione.

2. *Non-guideline 28 day dynamic/variable dosing study in Alpk:AP$_f$SD rats*

Male rats (20/group) were fed either control diets or variable dietary inclusion levels of mesotrione (100 to 0.3 ppm) for 28days. Two satellite groups (1 control and 1 variable dose treatment group) were included for measurement of plasma tyrosine and liver biochemistry.

Clinical observations, bodyweights and food consumption were measured throughout the study. The main phase animals were examined for ocular abnormalities pre-study and prior to termination. At scheduled termination, the animals were killed and examined macroscopically.

Dose rates (based on nominal dietary levels of mesotrione) were calculated in terms of mg mesotrione/kg bodyweight. The overall mean value was 2.39 mg mesotrione/kg/day (range: 0.025-13.03 mg mesotrione/kg/day). The received dose changed in line with dynamic dietary inclusion.

There was a slightly increased incidence of tail damage, scabs on the tail and scaly tail in the test group (group 2) compared to controls. However, in the absence of similar effects in the satellite group (group 4) the relationship to treatment with mesotrione is unclear. There were no treatment-related ocular lesions.

3. Non-guideline 28 day study in female Alpk:APfSD rats

Female rats (8/group) were fed diets containing mesotrione and/or tyrosine for 28 days, as follows:

Group 1. 0ppm mesotrione / 0% tyrosine
Group 2. 100ppm / 0%
Group 3. 100ppm / 0.5%
Group 4. 100ppm / 1.0%
Group 5. 100ppm / 2.5%
Group 6. 0ppm / 0.5%
Group 7. 0ppm / 1.0%
Group 8. 0ppm / 2.5%

Bodyweights and food consumption were recorded weekly. Ophthalmoscopy was performed prior to termination. Blood samples were taken for the assessment of plasma tyrosine levels at 24 hours, 1 week and prior to termination. Urine samples were assessed for ketone body content on Day 2, Day 8 and during the week prior to termination. Liver and kidney weights were recorded at necropsy, and liver samples (4 animals/dose group) were examined for tyrosine aminotransferase (TAT) and HPPD activity.

Mean bodyweights of Group 5 animals were significantly lower than controls from Week 3 (11.3%). Food consumption in this Group was significantly lower throughout the study period. Bodyweights of Group 3 and 4 animals were also lower than controls, however values did not attain statistical significance.

Significantly elevated plasma tyrosine concentrations were found in all groups administered mesotrione (Groups 2-5). Plasma levels in these groups increased with the dietary tyrosine concentration. Concentrations were also slightly increased in groups administered tyrosine alone, although values were considerably lower than equivalent groups administered tyrosine and mesotrione. TAT activity was significantly increased in all groups administered mesotrione and in Group 8 (0 ppm mesotrione + 2.5% tyrosine). Similarly, HPPD activity was significantly decreased in all groups administered mesotrione and in Group 8 (0ppm mesotrione with 2.5% tyrosine).

Cloudy eyes were seen in all animals of groups administered both tyrosine and mesotrione (Groups 3-5), with effects becoming apparent from Week 3-4. Ophthalmoscopy revealed corneal opacity and vascularisation in these animals related to the concentration of tyrosine administered. In animals dosed with mesotrione only, a single incidence of minimal corneal opacity was noted. Mean relative liver weights were significantly increased in all groups administered mesotrione and slightly (but significantly) in animals treated with 0.5% tyrosine only. Mean relative kidney weights were increased in groups administered mesotrione and tyrosine. Kidney and liver weights were slightly (but not significantly) increased in animals receiving 2.5% tyrosine.

Dietary tyrosine was shown to cause dose related increases in plasma tyrosine concentrations, with and without concurrent dosing with mesotrione. Dosing with 100 ppm of mesotrione resulted in HPPD inhibition and induction of TAT activity. The same effects were observed when dietary tyrosine was given at 2.5% in the absence of mesotrione. Corneal opacity and vascularisation were noted in groups receiving mesotrione, and the severity of ocular lesions in these groups increased with dietary tyrosine concentration. Increased liver weights were seen in groups administered

mesotrione and tyrosine, or mesotrione alone, and in animals administered with 0.5% tyrosine. Kidney weights were increased in groups administered both mesotrione and tyrosine.

Conclusions from the additional studies

These additional studies show that male rats fed a diet containing a certain level of tyrosine, develop ocular lesions which are ophthalmoscopically and microscopically similar to those seen in rats repeatedly dosed with mesotrione. In the 21 day study, these ocular lesions occurred at dietary tyrosine levels of ≥2.5% (w/w), and occurred with rapid onset (day 4 at 2.5% tyrosine, and day 3 at 5.0% tyrosine). Female rats are less sensitive to effects in the eyes, as demonstrated in the 28 day study. In this study, female rats fed diets of 2.5% tyrosine (without mesotrione) did not develop any eye lesions.

In the 28 day study in female rats, dosing with 100 ppm mesotrione and increasing levels of dietary tyrosine resulted in significantly elevated levels of plasma tyrosine. Plasma tyrosine levels increased with increasing dietary tyrosine concentration. TAT activity was significantly increased and HPPD activity was decreased in all groups administered mesotrione and in animals receiving 2.5% dietary tyrosine (without mesotrione).

In the dynamic feeding/variable dose study, no treatment-related ocular lesions occurred. This suggests that sustained levels of mesotrione are required in order for ocular lesions to develop in rats.

ii) Repeated dose toxicity by the oral route in mice

A guideline 1 year dietary study (which included a sacrifice after 90 days) and a guideline 80 week carcinogenicity study are available in mice. An additional non-guideline study which further investigates the effects seen in mice is also available. These studies are summarised in Table 12. Further information about the studies, based on the information in the RAR, is provided below the table.

Table 12: Summary table of relevant repeated dose toxicity studies by the oral route in mice

↑/↓ = increased/decreased compared to control. Unless otherwise stated, effects were seen in both sexes.

#The values for the NOAELs are provided for information only. All NOAEL values provided in this CLH Report have been taken from the Renewal Assessment Report (RAR), produced in accordance with Directive 91/414/EEC.

Method	Dose Levels	Significant toxicological findings
1 year dietary study with a sacrifice after 90 days Compliant with OECD 408 (minor deviations which do not effect reliability) and OECD 452 GLP compliant CD-1 mice, 20/sex/dose for 90 day study, 60/sex/dose for 1 year study Mesotrione (96.8% purity) in diet Anonymous (1997h) RAR B6.3.2.2	0, 10, 50, 350 and 700 ppm Equivalent to 0, 1.7, 8.4, 61.5, 1212 mg/kg bw/d in males and 0, 2.4, 12.4, 80.1 and 1537 mg/kg bw/d in females	**<u>Effects reported after 90 days:</u>** **10 ppm (1.7 / 2.4 mg/kg bw/d)** *Haematology & clinical chemistry* ↓ white blood cells (WBC) (males, 44.0 %), ↓ lymphocytes (males, 36.1%) , ↓ monocytes (males, 38.1%), ↓ eosinophil (males, 38.9%) **50 ppm (8.4 / 12.4 mg/kg bw/d)** *Haematology & clinical chemistry* ↓ WBC (males, 39.3%), ↓ lymphocytes (males, 39.6 %), ↓ monocytes (males, 51.1%), ↓ eosinophil (males, 54.0%) **350 ppm (61.5 / 80.1 mg/kg bw/d)** *Haematology & clinical chemistry* ↓ WBC (males, 40.1%), ↓ lymphocytes (males, 40.2%), ↓ monocytes (males, 49.7%), ↓ eosinophil (males, 56.6%) ↑ ALT (males, 31.1%) ↑ phosphorous (females, 21.5%) **7000 ppm (1212 / 1537 mg/kg bw/d)** *Haematology & clinical chemistry* ↓ WBC (males, 44.7%), ↓ lymphocyte (males, 46.7%), ↓ monocyte (males, 61.3%), ↓ eosinophil (males, 55.4%) ↑ ALT (males, 39.7%) ↑ phosphorous (males 25.7%, females 41%) *Ocular effects* Corneal opacity (2/12 males, cf. 0/12 in controls) Ruptured eye (1/12 males, cf. 0/12 in controls) Panophthalmitis (2/20 males, cf. 0/20 controls) **<u>Effects reported after 1 year</u>** **10 ppm (1.5 / 2.1 mg/kg bw/d)** *Clinical chemistry* ↑ urinary ketones (males) **50 ppm (7.8 / 10.3 mg/kg bw/d)** *Clinical chemistry* ↑ urinary ketones (males)

		350 ppm (56.2 / 72.4 mg/kg bw/d) *Clinical chemistry* ↓ creatinine (females, 10.9%) ↓ urine pH (males 6.5, cf. 7.0 in controls) ↑ urinary ketones (both sexes) **7000 ppm (1114 / 1494 mg/kg bw/d)** *Haematology* ↓ WBC (females, 47.2%) ↓ lymphocyte count (females, 53.4%) *Clinical chemistry* ↓ plasma urea levels (females, 38.0%) ↓ creatinine (males 5.4%, females 13.3%) ↓ AP (females, 26.8%) ↑ urine specific gravity (both sexes) ↓ urine pH (males 6.3, cf. 7.0 in controls) ↑ urinary ketones (both sexes) *Gall bladder* ↑ incidence and severity of eosinophilic change (females) *#A NOAEL of 350 ppm (equivalent to 56.2 and 72.4 mg/kg bw/d in males and females respectively) was identified in this study*
80 week carcinogenicity study in mice OECD 453, GLP compliant C57BL/10JfCD-1 mice (55/sex/dose level) Mesotrione (96.8% purity) in the diet Anonymous (1997i) RAR 6.5.2.2	0, 10, 350 and 7000 ppm Equivalent to 0, 1.4, 49.7 and 898 mg/kg bw/d in males and 0, 1.8, 63.5 and 1103 mg/kg bw/d in females	**<u>Non-neoplastic effects</u>** No significant non-neoplastic findings were observed in this study. *A NOAEL of 350 ppm (equivalent to 49.7 and 63.5 mg/kg bw/d in males and females respectively) has been determined for this study based on decreased bodyweight gain and food utilisation in males at 7000 ppm.*

90 day dietary response study

Non-guideline

GLP compliant

C57BL/10J$_f$AP/Alpk mice (10/sex/dose)

Mesotrione (96.8% purity)

Anonymous (1997j)

RAR: B6.8.2.10

0, 1, 10, 5, 100, 350, 1000, 3500 or 7000 ppm

Equivalent to 0, 0.16, 1.7, 8.5, 18, 59, 180, 600 or 1223 mg/kg bw/d in males and 0, 0.19, 1.9, 11, 21, 73, 215, 715 and 1436 mg/kg bw/d in females

Satellite groups (5/sex/dose) were terminated after 1 and 4 weeks

Biochemical parameters (all dose levels)

Plasma tyrosine concentrations (µM)

Wk	Sex	Dose group (ppm)								
		0	**1**	**12**	**50**	**100**	**350**	**1000**	**3500**	**7000**
1	**M**	125	186**	315**	483**	622**	506**	684**	639**	766**
	F	227	250	476**	591**	776**	750**	889**	986**	1251**
4	**M**	178	182	425**	524**	702**	722**	819**	957**	978**
	F	162	227**	423**	525**	530**	586**	681**	903**	960**
13	**M**	240	246	448**	285	533**	524**	726**	857**	837**
	F	181	168	305**	422**	454**	579**	659**	753**	749**

Free phenolic acids (mg eq/ml urine)

Wk	Sex	Dose group (ppm)								
		0	**1**	**12**	**50**	**100**	**350**	**1000**	**3500**	**7000**
13	**M**	1.91	nd	4.20	11.9	12.4	11.8	15.5	11.4	8.9
	F	nd	0.56	2.0	4.9	13.9	13.4	9.3	6.7	8.2

TAT activity (nM HPPA/min/mg protein)									
Wk	**Sex**	**Dose group (ppm)**							
		0	**1**	**12**	**50**	**100**	**350**	**1000**	**3500**	**7000**
1	**M**	9.6	9.2	9.5	10.7	12.4*	9.3	12.7*	7.3	8.7
	F	8.7	10.8	11.1	14.1**	13.5**	11.5	14.3**	12.0*	11.5
4	**M**	8.2	10.0	8.9	10.4	10.8	12.1**	9.6	7.4	9.2
	F	9.0	13.2*	14.3*	14.7**	16.9**	15.4**	15.6**	16.4**	18.4**
13	**M**	7.8	6.9	8.8	7.1	9.3	9.3	7.6	10.4*	9.6
	F	10.5	13.9	13.3	14.8*	17.1**	15.6	17.7*	15.4*	16.3**

HPPD activity (µl oxygen/min/mg protein)									
Wk	**Sex**	**Dose group (ppm)**							
		0	**1**	**12**	**50**	**100**	**350**	**1000**	**3500**	**7000**
1	**M**	0.404	0.145**	0.063**	0.046**	0.044**	0.054**	0.026**	0.050**	0.025**
	F	0.748	0.230**	0.091**	0.003**	0.031**	0.061**	0.046**	0.031**	0.024**
4	**M**	0.271	0.121**	0.042**	0.070**	0.061**	0.056**	0.054**	0.045**	0.009**
	F	0.293	0.120**	0.073**	0.122**	0.054**	0.051**	0.044**	0.026**	0.042**
13	**M**	0.071	0.036*	0.037*	0.046	0.026**	0.032*	0.018**	0.020**	0.006**
	F	0.150	0.102	0.128	0.095	0.059*	0.060*	0.054**	0.046**	0.038**

Combined guideline 90 day and 1 year repeated dose toxicity studies in CD-1 mice

A guideline 1 year chronic toxicity study is available in CD-1 mice in which a subset of animals was sacrificed after 90 days, and the results reported as a guideline 90 day repeated dose toxicity study. Animals (20/sex/dose for the 90 day study and 60/sex/dose for the 1 year study) were administered mesotrione in the diet at 0, 10, 50, 350 or 7000 ppm. 20 animals/sex/dose in the one year study were sacrificed at 6 months.

Significant toxicological effects reported after 90 days

The target organs were the eyes. Ophthalmoscopy revealed a slightly increased incidence of corneal opacity in males at 1212 mg/kg bw/d; one of these males also had a ruptured eye. Panophthalmitis was noted in 2 males at the top dose (compared to 0 in controls).

Total white blood cell, lymphocyte and monocyte counts were significantly decreased in all treated groups of males. Neutrophil counts were also reduced in all groups of treated males. The toxicological significance of the white blood cell findings in males is unclear in the absence of similar findings in females or at later time points. The applicant proposes that decreases in white blood cell parameters are due to stress-induced leucocytosis. Eosinophil numbers were significantly decreased in all treated groups of males and decreased in all treated groups of females (significant only at 80.1 mg/kg bw/d).

(i) Significant toxicological effects reported after one year

Slight increases (<10%) in liver and kidney weights were noted in both sexes at 7000 ppm. No macroscopic findings attributable to treatment were noted at necropsy in either organ. Treatment-related microscopic findings were limited to an increase in the incidence and severity of eosinophilic change of the gall bladder epithelium in females at 1494 m/kg bw/d. In the absence of additional microscopic findings, the toxicological significance of this finding (reported to be common in this strain) is unclear.

Guideline 18 month carcinogenicity study in CD-1 mice

Mice (55/sex/dose level) were administered mesotrione (96.8% purity) in the diet at 0, 1.4, 49.7 and 898 mg/kg bw/d in males and 0, 1.8, 63.5 and 1103 mg/kg bw/d in females. Top dose animals were initially administered 3500 ppm mesotrione, this was increased to 7000 ppm in Week 7.

Weight gain and food utilisation was decreased in males at 898 mg/kg bw/d, and terminal body weights were slightly lower (↓7.4%) than controls. No effects were seen in females.

No significant non-neoplastic findings were observed in this study.

90 day dietary response study in C57BL/10J$_{\beta}$AP/Alpk mice

In a non-guideline study, mice (10/sex/dose level) were fed diet containing mesotrione at 0, 0.16, 1.7, 8.5, 18, 59, 180, 600 and 1223 mg/kg bw/d (males) and 0, 0.19, 1.9, 11, 21, 73, 215, 715 and 1436 mg/kg bw/d (females) for 90 days. Satellite groups (5/sex/dose level) were terminated after 1 and 4 weeks. Bodyweights and food consumption were recorded weekly. Terminal blood samples were analysed for plasma tyrosine concentrations. Overnight urine samples were analysed by NMR for phenolic acid content. Kidneys and liver were weighed at necropsy and examined for TAT and HPPD activity (5/dose level). Full histopathological examinations were performed on 4 mice/sex/dose level.

In both sexes, there was a dose-related increase in plasma tyrosine levels. After 1 week, plasma tyrosine levels were higher in females than in males at all dose levels. After 4 and 13 weeks, this trend had reversed and tyrosine levels were generally higher in males. Hepatic TAT activity was increased in females at all treatment levels and at all time points. TAT activity was increased in some groups of treated males but with no clear dose-response relationship. At some dose/time points, TAT activity in females was more than double that in males. HPPD activity was significantly decreased in all groups of treated mice in a dose-related manner.

Urinary phenolic acids were increased in males ≥10 ppm and in all groups of treated females. Phenolic acids were characterised by NMR as HPLA, HPAA and HPPA.

In summary, administration of mesotrione caused a dose-related hypertyrosinaemia in all groups of treated mice. Hypertyrosinaemia correlated with the presence of phenolic acid metabolites of tyrosine (HPLA, HPAA and HPPA) in the urine of treated animals. A dose-related inhibition of HPPD activity was noted in all groups of treated mice. Induction of TAT activity was also apparent, although there was no clear dose-response relationship.

iii) Repeated dose toxicity by the oral route in dogs

Two guideline studies are available which investigate the repeated dose toxicity of mesotrione in dogs by the oral route (a 90 day study and a 1 year study). These studies are summarised in Table 13. Further information about the studies, based on information in the RAR, is provided below the table.

Table 13: Summary table of standard repeated dose toxicity studies by the oral route in dogs

↑/↓ = increased/decreased compared to control. Unless otherwise stated, effects were seen in both sexes.

#The values for the NOAELs are provided for information only. All NOAEL values provided in this CLH Report have been taken from the Renewal Assessment Report (RAR), produced in accordance with Directive 91/414/EEC.

Method	Dose Levels	Significant toxicological findings
90 day oral study OECD 408 with slight deviations – see text GLP compliant Beagle dogs, 4/sex/dose Mesotrione (96.8% purity) in capsules Anonymous (1997k) RAR B 6.3.2.3.1	0, 100, 600 and 1000 mg/kg bw/d	**100 mg/kg bw/d** No significant findings **600 mg/kg bw/d** *Haematology* ↑ RBC (males from week 8, 11.7-16.2%, females at week 13, 16.5%) ↓ MCV (from week 4, males 3.8-14.0%, females 2.8-12.0% ↓ MCH (males from week 4, 4.4 - 13.2%, females from week 8, 7.0 – 12.6%) ↑ platelets (females, 26.5%) ↓ lymphocytes (males at week 13, 23.7%, females from week 4, 19.8 - 21.2%) **1000 mg/kg bw/d** *Haematology & clinical chemistry* ↑ RBC (males from week 4, 11.6 – 16.2%, females from week 8, 11.8-19.7%) ↓ MCV (from week 4, males 5.3 - 16.2%, females 3.3 -13.6%) ↓ MCH (males from week 4, 6.2 - 15.9%, females from week 8, 7.8 - 12.6%) ↑ platelets (males, 26.5%) ↓ lymphocytes (males at 13 weeks, 23.4%, females from 4 weeks, 23.1-29.5%) ↓ APTT (females 10.6%) ↓ urea (males from week 4, 40.2 - 46.8%) *Organ weights* ↓ relative brain weight (females, 11.5%) *Microscopy findings* Minimal or slight mesothelial proliferation of the atrium of the heart (2/4 males, cf. 0/4 in controls) *#NOAEL = 100 mg/kg bw/d based on haematological changes indicative of microcytic polycythaemia in both sexes at ≥ 600 mg/kg bw/d*

1 year oral study OECD 452 with slight deviations – see text GLP compliant Beagle dogs, 4/sex/dose Mesotrione (97.6% purity) in capsules Anonymous (1997l) RAR B6.3.2.3.2	0, 10, 100 and 600 mg/kg bw/d	**10 mg/kg bw/d** *Clinical chemistry* ↑ tyrosine (males 168%, females 151%) **100 mg/kg bw/d** *Clinical chemistry* ↑ triglycerides (females, 107.4%) ↑ tyrosine (males 708%, females 1213%) *Lungs* Firm nodules (2/4 females) *Kidneys* ↑ weight (males 32.0%) **600 mg/kg bw/d** *Mortality* One animal humanely killed at 47 weeks *Haematology* ↓ MCV (females, 3.1 – 10.3%) ↓ MCH (females, 2.7 – 11.9%) *Clinical chemistry* ↓ cholesterol (males, 19.4%) ↓ bilirubin (males 38.6 – 43.4%, females 6.0 -31.7%) ↑ ALT (females, 151%) ↑ phosphorous (males 8.7 - 25%, females 27.4 - 30.6%) ↑ tyrosine (males 915%, females 1470%) *Ophthalmoscopy* Lenticular opacity (1/4 males) Corneal opacity (1/4 males) Corneal keratitis (1/4 males) *Lungs* Firm nodules (2/4 females) *#NOAEL = 100 mg/kg bw/d based on signs of systemic toxicity, corneal opacity and microscopic findings at 600 mg/kg bw/d*

Guideline 90 day repeated dose toxicity study in beagle dogs

Dogs (4/sex/dose level) were administered capsules of mesotrione at 0, 100, 600 or 1000 mg/kg bw/day. Ophthalmoscopy was performed pre-test and after 12 weeks of treatment.

Dose-related increases in red blood cell count and decreases in MCV and MCH were noted in both sexes from Week 4. The magnitude of these findings increased with time and values attained statistical significance at 600 and 1000 mg/kg bw/d. Platelet numbers were increased in all treated animals (statistically significant in males at the top dose and females at 600 mg/kg bw/d). Lymphocyte numbers in treated animals of both sexes were reduced throughout the study, values attained statistical significance at 600 and 1000 mg/kg bw/d at 13 weeks and in females at 4 weeks only.

At necropsy, mean relative brain weights were significantly reduced in top dose females (11.5%), largely due to one animal. Mean relative liver weight was significantly higher in top dose males (<10%). The only microscopic finding was minimal or slight mesothelial proliferation of the atrium of the heart in two male dogs at 1000mg/kg/d. No other findings attributable to treatment were noted.

Guideline 1 year repeated dose toxicity study in beagle dogs

Dogs (4/sex/dose) were administered capsules of mesotrione at 0, 10, 100 or 600 mg/kg bw/day for one year. Veterinary examinations (including ophthalmoscopy) were performed during Weeks 13, 29, 39 and prior to termination. Additional blood samples were taken at termination for the assessment of plasma tyrosine levels.

One female in the 600 mg/kg bw/d group was humanely killed during Week 47 after exhibiting rapid weight loss, convulsions, hypothermia and a slow pulse. No further deaths occurred during the study.

Plasma tyrosine concentrations at termination were significantly increased in all groups of treated animals. Urinalysis revealed slightly increased levels of ketones in both sexes at 100 and 600 mg/kg bw/d at Week 26 and 52. NMR analysis of urine from 2 animals/sex/dose level showed high levels of free and total phenolic acids in treated animals. Levels of conjugated phenolic acids were not affected by treatment. Free phenolic acids were identified as 4-hydroxyphenyl pyruvate and 4-hydroxyphenyl lactate.

In the eyes, ophthalmoscopy revealed single incidences of lenticular opacity in both sexes at 600 mg/kg bw/d. At scheduled necropsy, corneal and lenticular opacity and corneal keratitis were observed in one male at this dose.

Kidney weights of 100 mg/kg bw/d males were significantly greater than controls (↑32%), however there was no clear dose-response and kidney weights at the top dose were not significantly different to controls.

4.7.1.2 Repeated dose toxicity: inhalation

No data are available.

4.7.1.3 Repeated dose toxicity: dermal

One standard study (conducted according to guidelines) is available which investigates the repeated dose toxicity of mesotrione in rabbits by the dermal route. This study is summarised in Table 14. Further information about the study, based on information in the RAR, is provided below the table.

Table 14: Summary table of standard repeated dose toxicity studies by the dermal route in rabbits

↑/↓ = increased/decreased compared to control. Unless otherwise stated, effects were seen in both sexes.

#The values for the NOAELs are provided for information only. All NOAEL values provided in this CLH Report have been taken from the Renewal Assessment Report (RAR), produced in accordance with Directive 91/414/EEC.

Method	Dose Levels	Observations and Remarks
21 day dermal study OECD 410 GLP compliant New Zealand white rabbits, 5/sex/dose Mesotrione (purity 96.8%) made to a paste with deionized water Anonymous (1997m) RAR B 6.3.3.1	0, 10, 500 or 1000 mg/kg bw/d	**1000 mg/kg bw/d** *Haematology & clinical chemistry* ↑ RBC distribution width (females, 14.9%) ↑ Cholesterol (females, 33%) ↑ CK (females, 229%) Nb. Haematological and clinical chemistry parameters for male animals were not reported due to a high incidence of haemolysis in terminal blood samples. The phenomenon was attributed to the presence of 'Euthatal' in the blood. *#NOAEL = 1000 mg/kg bw/d, based on the absence of any clear treatment-related effects. However, the limitations of this study are recognised.*

Guideline 21 day study in New Zealand white rabbits

Rabbits (5/sex/dose level) were administered mesotrione (6 hour dermal applications) at 0, 10, 500 or 1000 mg/kg bw/d. Ophthalmoscopy was performed pre-test and prior to termination.

Slight erythema at the application site was noted in all top dose animals and in one male and three females at 500 mg/kg bw/d. All signs of skin irritation had regressed by the end of the study. Ophthalmoscopy did not reveal any treatment-related findings.

4.7.1.4 Repeated dose toxicity: other routes

No data are available.

4.7.1.5 Human information

The pharmacokinetic and pharmacodynamics of mesotrione have been investigated in humans. Further information regarding the proposed mode of action is available from the administration of NTBC (nitisinone) to humans to treat Type I tyrosinaemia and alkaptonuria. This information is summarised below.

***Human Volunteer Study with Mesotrione and NTBC* (2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione**

The pharmacokinetic and pharmacodynamics of mesotrione have been investigated in healthy male volunteers (Hall *et al*, 2001). In the study, three groups of 6 volunteers were given a single dose of either 0.1, 0.5 or 4 mg/kg bw mesotrione. A series of blood and urine samples were taken up to 96 hours after dosing. Plasma was analysed for the presence of mesotrione and tyrosine.

No treatment-related adverse effects were observed in any of the volunteers. At each dose, peak plasma concentrations of mesotrione were observed within 6 hours of dosing and had declined to below the limit of quantification of the assay within 12 hours of dosing. The estimated half time of mesotrione in plasma was approximately 1 hour and was independent of dose. Unchanged mesotrione was rapidly excreted in the urine at each dose investigated with the majority of the recovered dose recovered within 12 hours of dosing. The mean concentrations of tyrosine in the plasma from each group of volunteers prior to dosing showed considerable inter-individual and temporal variation (values tended to be higher during the day than during the night). At each dose level of mesotrione, the mean peak concentrations of tyrosine in plasma during the 24 hours following dosing were higher than those in the 24 hours pre-dosing. The increase in mean peak tyrosine concentrations were transient, apart from in those volunteers receiving 4 mg/kg bw/d where the effects persisted beyond 24 hours. In these volunteers, tyrosine levels returned to pre-dose levels within 48 hours of dosing. Urinary excretion of tyrosine metabolites was increased during the 24 hours immediately following the dose of 4 mg/kg bw/d (but not lower doses), and returned to background levels during the following 24 hours.

In a separate part of the study, ten volunteers (two groups of five) were each given two separate oral doses of 1 mg/kg bw NTBC (a triketone which is used therapeutically in humans to treat hereditary hypertyrosinaemia type 1) 14 days apart. Following administration, no treatment-related adverse effects were observed. Peak plasma concentrations of NTBC (~1100 nmol/ml) were rapidly obtained and the half-life in plasma was estimated to be 54 hours. Concentrations were still approximately 8 times those of background levels 14 days after dosing, but had returned to background levels within 2 months of the second dose. When normalised for dose, the extent of the induced hypertyrosinaemia following dosing with NTBC was 400 fold greater than that following administration of mesotrione.

Repeated Dosing with NTBC and Plasma Tyrosine Levels in Humans

In a study investigating the metabolic effects of increasing doses of NTBC in the treatment of alkaptonuria, patients were given 1, 2, 4, 6 or 8 mg/kg bw/d of NTBC for a minimum of 6 months. Plasma tyrosine levels were shown to plateau at around 650-700 nmol/ml in the patients dosed with 2 mg/kg bw/d of NTBC, and did not increase in those patients receiving higher doses (Gertsman *et al.,* 2015).

Given that NTBC is a more potent inhibitor of HPPD than mesotrione, it can be expected that plasma tyrosine levels will not exceed this value in humans repeatedly dosed with mesotrione.

4.7.1.6 Other relevant information

No other relevant information.

4.8 Specific target organ toxicity – repeated exposure (STOT RE)

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE

Oral Route

The repeated dose toxicity has been investigated in rats, mice and dogs. Some evidence is also available from a human volunteer study.

The eyes and kidneys were identified as the target organs in rats, whereas only the eyes where affected in mice and dogs.

Effects in the eye

The eyes were a target organ in all 3 species, with the rat being the most sensitive (and male rats more sensitive than female rats). In rats, effects in the eye consisted of ocular opacity as a result of corneal keratitis, epithelial disruption and associated vascularisation, and were seen from doses ≥ 0.71 mg/kg bw/d in males (guideline 90 day study). Eye effects were not observed in the 28 day study, but were noted from 1 week of exposure in a non-guideline study to investigate ocular toxicity development and reversibility in rats. In non-guideline reversibility studies in rats, the effects in the eye were found to be reversible following cessation of treatment. In mice, corneal opacity was noted at low incidence (2/12 males) at the top dose (1212 mg/kg bw/d) in the guideline 90 day study. In dogs, lenticular opacity, corneal opacity and corneal keratitis were noted in 1/4 males at the top dose (600 mg/kg bw/d) in the 1 year chronic toxicity study.

In the MoA proposed by the applicant (Annex 1), the effects in the eye are said to be due to hypertyrosinaemia. This is supported by a non-guideline 21 day study looking at the effects of dietary tyrosine on ocular lesions in rats. In this study, rats administered high concentrations of tyrosine in a low protein diet rapidly developed ocular lesions which were ophthalmoscopically and microscopically identical to those seen in rats administered mesotrione. Data taken from a series of studies in which groups of rats were dosed for 6 weeks with different triketone HPPD inhibitors showed that corneal lesions only occurred once plasma tyrosine levels exceeded 1000 nmol/ml (see Figure 8-5, Annex 1).

Species differences in the susceptibility to eye effects are said to be due to the degree of hypertyrosinaemia and therefore due to species differences in TAT activity. As indicated in Section 4.7.16, the innate TAT activity in humans is similar to that in mice, significantly higher than in rats.

The RAC conclusion for tembotrione noted that NTBC has been shown to greatly increase tyrosine concentrations in healthy adult volunteers treated with a single dose of 1 mg/kg bw/day NTBC and to cause eye problems in some children treated with 1 mg/kg bw/day NTBC (against Type 1 hypertyrosinaemia). Therefore, there is an intrinsic possibility that mesotrione could cause similar effects in humans. However, data are available from a human volunteer study using mesotrione which demonstrate it is ~400 times less potent than NTBC. Therefore, effects in the eyes are not expected to occur in humans following exposure to mesotrione at doses relevant for classification.

Effects in the kidney

The kidney was a target organ in rats only, with males being more sensitive than females. A number of effects were seen in the available repeated dose toxicity studies (subchronic and chronic) and in a guideline multigeneration study in rats (Section 4.11). The effects generally lacked a clear dose-response and were inconsistent across studies of different durations. However, whilst there are inconsistencies in the findings observed across the different studies, it is difficult to dismiss the effects entirely (especially the renal pelvic dilatation and the chronic progressive glomerulonephropathy). It is likely that these effects are associated with the tyrosinaemia, but definitive evidence is not available to demonstrate that this is the case. Furthermore, there is no evidence regarding the relevance of the effect in humans.

Inhalation Route

No data are available.

Dermal Route

In a guideline 21 day dermal study in NZW rabbits, no adverse effects were observed in either sex at doses ≤ 1000 mg/kg bw/d.

4.8.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

Oral Route

Classification for STOT-RE 2 is assigned on the basis of findings of 'significant' or 'severe' toxicity at dose levels ≤ 100 mg/kg bw/day (guidance value, based on a 90-day study in rats and adjusted for study duration accordingly). Classification for STOT-RE 1 is assigned on the basis of findings of 'significant' or 'severe' toxicity at dose levels ≤ 10 mg/kg bw/day (guidance value, based on a 90-day study in rats and adjusted for study duration accordingly).

Following repeated exposure to mesotrione, significant effects were observed in the eyes and kidneys. The effects in the eyes are considered to be of significant concern and were observed at doses below the relevant guidance values for classification (i.e., from 0.71 mg/kg bw/d in male rats in a guideline 90 day study). However, data are available from human studies in which individuals received either mesotrione or NTBC (a triketone which is used therapeutically in humans to treat hereditary hypertyrosinaemia type 1). These studies provide evidence to show that mesotrione is ~400 times less potent than NTBC and eye effects are not expected to occur in humans at doses below those relevant for classification. Therefore, these effects do not support classification for STOT-RE.

The effects in the kidneys were inconsistent, but overall are considered to be of significant concern. It is possible that these effects could be due to tyrosinaemia, but definitive evidence is not available to demonstrate that this is the case. Furthermore, there is no evidence regarding the relevance of the effect in humans and, therefore, they cannot be dismissed. The chronic progressive glomerulonephropathy was observed in a 90-day oral rat study at a dose level of 14.5 mg/kg bw/day. Effects were noted at lower dose levels in the 2-year carcinogenicity study (from 0.48 mg/kg bw/day) and the multigeneration study but are difficult to interpret given the variation in the nature and severity of the findings. Overall, it is proposed that these available data support classification for **STOT-RE 2; H373 – May cause damage to organs (kidneys) through prolonged or repeated exposure**.

Inhalation Route

No data are available

Dermal Route

Under CLP, the guidance value for classification in STOT RE Category 2 in a 28 day dermal study in rats is ≤ 600 mg/kg bw/d.

In a guideline 21 day dermal study in white New Zealand rabbits, no adverse effects were observed in either sex at doses ≤ 1000 mg/kg bw/d. Therefore, the criteria for classification are not met.

4.8.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

STOT-RE 2; H373 – May cause damage to organs (kidneys) through prolonged or repeated exposure.

4.9 Germ cell mutagenicity (Mutagenicity)

Mutagenicity has been investigated in three guideline *in vitro* studies and one guideline *in vivo* study. These studies are summarised in Table 15.

Table 15: Summary table of relevant in vitro and in vivo mutagenicity studies

In Vitro Data			
Method	**Organism/strain**	**Concentrations tested**	**Result**
Bacterial reverse mutation test OECD 471 GLP compliant Mesotrione (96.6% purity) Harlan Cytotest Cell Research GmBH (2013) RAR B.6.4.1	*S. typhimurium:* TA1535 TA1537 TA98 TA100 *E. coli:* WP2*urvA* pKM101 WP2 pKM101	0, 3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate ± S9	Negative
Cytogenetic assay in human lymphocytes OECD 473 GLP compliant Mesotrione (98.1% purity) Zeneca Central Toxicology Laboratory (1994c) RAR B.6.4.1	Human lymphocytes	250 – 2000 µg/ml ± S9	Equivocal
Mammalian cell gene mutation test OECD 476 GLP compliant Mesotrione (98.1% purity) Zeneca Central Toxicology Laboratory (1994d) RAR B.6.4.1	Mouse lymphoma L5178Y	125 – 1000 µg/ml	Negative

In vivo Data			
Method	**Organism/strain**	**Concentrations tested**	**Result**
Micronucleus test OECD 474 Mesotrione (98.1% purity) Anonymous (1994c) RAR: B.6.4.2	CD-1 mice (5/sex/sample time)	500 mg/kg bw in deionised water. The dose level was based on the results of a preliminary study in which 8/10 animals dosed with 800 mg/kg bw (the next highest dose level) died. No specific evidence of bone marrow exposure was obtained.	Negative

4.9.1 Non-human information

4.9.1.1 In vitro data

A guideline bacterial reverse mutation assay is available. No evidence of mutagenicity was observed in the test. Additional bacterial reverse mutation assays are included in the RAR, however they are not considered here as they used an old specification of mesotrione technical material.

A guideline cytogenetic assay in human lymphocytes is available. In the study, the potential clastogenicity of mesotrione was investigated in cultured lymphocytes from two human donors. Concentrations of 250-2000 μg/ml were used (based on the limit of solubility, cytotoxicity and pH effects) in the presence and absence of a metabolic activation system (phenobarbital/β-naphthoflavone induced rat liver S9). Cells were incubated for 48 hours prior to the addition of test material. One hundred metaphase cells per suitable culture were examined at 68 hours (both donors) and 92 hours (donor 2 only).

A dose related increase in the number of aberrant cells (excluding cells with gaps only) was seen at the 68 hour sample time in cultures in the absence of S9 from one donor. Values at 1000 and 1500 μg/ml mesotrione attained statistical significance but were within the historical control range. No such increase was seen in cultures of lymphocytes from the other donor at either the 68 or 92 hour sampling times. Single positive control cultures (mitomycin C and cyclophosphamide) produced significant increases in the number of cells with chromosomal aberrations.

No reproducible, statistically significant increase in the number of aberrant cells was seen in this study. However in view of the positive findings in one donor, the results of this study are considered to be equivocal.

A guideline mammalian cell gene mutation test is available. No evidence of mutagenicity was observed in the study.

4.9.1.2 In vivo data

A guideline micronucleus study is available in CD-1 mice. No evidence of clastogenicity was observed in this study.

4.9.2 Human information

No data are available.

4.9.3 Other relevant information

N/a.

4.9.4 Summary and discussion of mutagenicity

A number of *in vitro* tests (a bacterial reverse mutation test, a cytogenetic assay in human lymphocytes and a mammalian cell gene mutation assay) and an *in vivo* micronucleus assay are available. There was no indication of gene mutation either in the presence or absence of metabolic activation in both the bacterial reverse mutation and mammalian gene mutation tests. An equivocal result was obtained in the cytogenetic assay using human lymphocytes, however an *in vivo* mouse micronucleus test was negative. Based on these studies, it can be concluded that mesotrione has no genotoxic potential.

4.9.5 Comparison with criteria

Based on these studies, it is concluded that mesotrione is not genotoxic. Therefore, classification is not warranted.

4.9.6 Conclusions on classification and labelling

Not classified – conclusive but not sufficient for classification

4.10 Carcinogenicity

Carcinogenicity has been investigated in two guideline studies – one in rats and one in mice. These studies are summarised in Table 16.

Table 16: Summary table of relevant carcinogenicity studies

↑/↓ = increased/decreased compared to control. Unless otherwise stated, effects were seen in both sexes.

#The values for the NOAELs/NOELs are provided for information only. All NOAEL/NOEL values provided in this CLH Report have been taken from the Renewal Assessment Report (RAR), produced in accordance with Directive 91/414/EEC.

<table>
<tr><th>Method</th><th>Dose levels</th><th>Observations and remarks
(effects of major toxicological significance)</th></tr>
<tr><td>2 year dietary toxicity and oncogenicity study in rats

OECD 453

GLP compliant

Alpk:AP$_f$SD rats (64/sex/dose level)

Mesotrione (96.8% purity) in the diet
Anonymous (1997n)
RAR B.6.5.1.1</td><td>0, 1, 2.5, 7.5, 100 and 2500 ppm

Equivalent to:
0.0, 0.06, 0.16, 0.48, 6.5 and 160 mg/kg bw/d in males

0.0, 0.09, 0.19, 0.57, 7.7 and 190 mg/kg bw/d in females</td><td>Non-neoplastic effects
See Table 10, Section 4.7 repeated dose toxicity

Neoplastic Effects
<table>
<tr><th rowspan="2">Tumour Type</th><th colspan="5">Tumour incidence (%)</th></tr>
<tr><th>0 ppm</th><th>7.5 ppm</th><th>100 ppm</th><th>2500 ppm</th><th>Historical control</th></tr>
<tr><td colspan="6">Males</td></tr>
<tr><td>Total malignant</td><td>20.3</td><td>14.1</td><td>12.5</td><td>9.4</td><td>--</td></tr>
<tr><td>Total benign</td><td>50.0</td><td>32.8</td><td>46.9</td><td>39.1</td><td>--</td></tr>
<tr><td>Hepatocellular adenoma</td><td>3.1</td><td>1.6</td><td>1.6</td><td>0.0</td><td>0-6.7
(2.3)</td></tr>
<tr><td>Thyroid follicular adenoma</td><td>0.0</td><td>1.6</td><td>4.7</td><td>1.6</td><td>0-11.5
(2.4)</td></tr>
<tr><td colspan="6">Females</td></tr>
<tr><td>Total malignant</td><td>28.1</td><td>40.6</td><td>35.9</td><td>23.4</td><td>--</td></tr>
<tr><td>Total benign</td><td>76.6</td><td>79.7</td><td>75.0</td><td>71.9</td><td>--</td></tr>
<tr><td>Hepatocellular adenoma</td><td>0.0</td><td>3.1</td><td>6.3</td><td>1.6</td><td>0-3.9
(1.0)</td></tr>
<tr><td>Thyroid follicular adenoma</td><td>0.0</td><td>1.6</td><td>1.6</td><td>6.3</td><td>0-3.9
(0.8)</td></tr>
</table>
A #NOEL for neoplastic change of 100 ppm (equivalent to 7.7 mg/kg bw/d) has been determined for this study based on an increased incidence of thyroid follicular adenoma in females at 2500 ppm.</td></tr>
</table>

80 week carcinogenicity study in mice OECD 453 GLP compliant C57BL/10J$_f$CD-1 mice (55/sex/dose level) Mesotrione (96.8% purity) in the diet Anonymous (1997o) RAR 6.5.2.2	0, 10, 350 and 7000 ppm Equivalent to 0, 1.4, 49.7 and 898 mg/kg bw/d in males and 0, 1.8, 63.5 and 1103 mg/kg bw/d in females	***Neoplastic effects*** *No significant non-neoplastic effects were observed during this study* ***Non-neoplastic effects*** No significant non-neoplastic findings were observed in this study *A NOAEL of 350 ppm (equivalent to 49.7 and 63.5 mg/kg bw/d in males and females respectively) has been determined for this study based on decreased bodyweight gain and food utilisation in males at 7000 ppm.*

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

Guideline 2 year carcinogenicity study in Alpk:AP$_f$SD rats

Rats (64/sex/dose level) were administered mesotrione in the diet at 0.0, 0.06, 0.16, 0.48, 6.5 and 160 mg/kg bw/d in males and 0.0, 0.09, 0.19, 0.57, 7.7 and 190 mg/kg bw/d in females for up to 104 weeks. 12 animals/sex/dose were scheduled for termination at 52 weeks. Two additional groups (20/sex) were administered mesotrione at 1.0 or 2.5 ppm for the investigation of ocular toxicity. Non-neoplastic findings are discussed in Section 4.7 repeated dose toxicity.

Groups were terminated when survival reached 25%. Survival of groups dosed at 1 ppm and 2.5 ppm was low, however survival of males at ≥7.5 ppm was 31% at 96 weeks. Survival in all groups of females was ≥50% at scheduled termination. It was concluded that the survival of animals and the duration of exposure in this study permitted adequate assessment of the carcinogenic potential of mesotrione. Male survival rate is shown in the table below.

Dose Group		**Kaplan-Meier % Survival Rate at Week**							**Terminated**
(ppm)		**80**	**84**	**88**	**92**	**96**	**100**	**104**	(at Week)
M	**0**	71	65	61	52	46	--	--	**97**
	1.0	50	45	35	30	--	--	--	**92**
	2.5	60	50	35	25	--	--	--	**92**
	7.5	63	57	53	37	31	--	--	**97**
	100	72	66	53	35	31	--	--	**97**
	2500	65	57	46	36	31	--	--	**97**

The incidence of hepatocellular adenoma was increased in treated females and was outside the historical control range at 6.5 mg/kg bw/d. However, in the absence of a clear dose-response relationship, this finding is not considered to be attributable to treatment with mesotrione.

The incidence of thyroid follicular cell adenoma was slightly increased in treated groups of both sexes and was outside the historical range in top dose females. The incidence of thyroid follicular cell carcinoma was 0, 1, 0 and 1 in males at 0, 0.48, 6.5 and 160 mg/kg bw/d respectively and was within the HCD of the laboratory (incidence 0 – 1 (0-2%)). No incidences of follicular cell carcinoma were observed in females. The incidence of cystic/hyperplastic thyroid was also increased in this group, The effects consisted of a slight increase in the incidence of squamous cysts in females (0, 1, 2 and 5 at 0, 0.57, 7.7 and 190 mg/kg bw/d), a slight increase in the incidence of follicular cysts in males (1, 0, 3 and 5 at 0, 0.48, 6.5 and 160 mg/kg bw/d) and an increased incidence of follicular cysts with hyperplastic epithelium (1, 5, 7 and 5 at 0, 0.48, 6.5 and 160 mg/kg bw/d in males and 0, 0, 1, 3 at 0, 0.57, 7.7 and 190 mg/kg bw/d in females). No follicular hyperplasia was observed in either sex.
The study authors noted that the overall tumour burden in treated females was similar to controls, and concluded that mesotrione was not carcinogenic in the study.

2 year guideline carcinogenicity study in C57BL/10J$_f$CD-1 mice

Mice (55/sex/dose level) were administered mesotrione (96.8% purity) in the diet at 0, 10, 350 or 7000 ppm for 80 weeks (equivalent to 0, 1.4, 49.7 and 898 mg/kg bw/d in males and 0, 1.8, 63.5 and 1103 mg/kg bw/d in females). Top dose animals were initially administered 3500 ppm mesotrione, this was increased to 7000 ppm in Week 7. Weights of the adrenals, brain, kidneys, liver and testes were recorded at necropsy. A comprehensive list of tissues from each animal was examined microscopically.

Non-neoplastic effects are discussed in Section 4.7 Repeated Dose Toxicity.

No evidence of carcinogenicity was seen in this study, and no target organs were identified.

4.10.1.2 Carcinogenicity: inhalation

No data are available.

4.10.1.3 Carcinogenicity: dermal

No data are available.

4.10.2 Human information

No data are available.

4.10.3 Other relevant information

No other relevant data are available.

4.10.4 Summary and discussion of carcinogenicity

Carcinogenicity has been investigated in two guideline studies – one in rats and one in mice. Non-neoplastic findings are discussed in Section 4.7 – repeated dose toxicity.

The incidence of thyroid follicular cell adenoma was slightly increased in top dose females and was outside the historical control range (incidences 0, 1.6, 1.6, 6.3% at 0, 0.57, 7.7 and 190 mg/kg bw/d; female historical control range 0 - 3.9%). The incidence of cystic/hyperplastic thyroid was also increased and it could be considered that there is a treatment related effect. However, the same findings of cystic/hyperplastic thyroid were also observed in males, but there was no increase in tumours. Therefore, overall, it cannot be concluded that the findings in female rats are a treatment related effect.

In the mouse study, no neoplastic findings or effects in the thyroid were observed.

In the mutagenicity studies, there was no evidence for a genotoxic potential of mesotrione.

4.10.5 Comparison with criteria

Overall, there is no clear evidence of treatment-related tumours in rats or mice. Therefore, no classification is proposed.

4.10.6 Conclusions on classification and labelling

Not classified – conclusive but not sufficient for classification

4.11 Toxicity for reproduction

4.11.1 Effects on fertility

Fertility effects have been investigated in a guideline multigeneration study in rats, a non-guideline single generation study in rats and a guideline multigeneration study in mice. These studies are summarised in Table 17, over the page. Further information about the studies, taken from the RAR, is provided below the table.

Table 17: Summary table of relevant reproductive toxicity studies – Fertility

↑/↓ = increased/decreased compared to control. Unless otherwise stated, effects were seen in both sexes.

*significantly different to controls (p<0.05), ** p<0.01

#The values for the NOAELs are provided for information only. All NOAEL values provided in this CLH Report have been taken from the Renewal Assessment Report (RAR), produced in accordance with Directive 91/414/EEC.

<table>
<tr><th>Method</th><th>Dose levels</th><th>Observations and remarks
(effects of major toxicological significance)</th></tr>
<tr><td>Multigeneration study in the rat

OECD 416

GLP compliant

Alpk:AP_fSD rats (26/sex/dose level)

Mesotrione (96.8% purity)

Anonymous (1997p)

RAR B.6.6.1.1</td><td>0, 2.5, 10, 100 or 2500 ppm

Equivalent to 0, 0.2-0.6, 0.9-2.3, 9.0-21.7 and 236-476 mg/kg bw/d, depending on sex, generation and period of study (pre-mate, gestation or lactation)</td><td><u>Reproductive and developmental parameters</u>

<table>
<tr><th>Parameter</th><th>Generation</th><th colspan="5">Dose Level (ppm)</th></tr>
<tr><th></th><th></th><th>0</th><th>2.5</th><th>10</th><th>100</th><th>2500</th></tr>
<tr><td rowspan="4">Gestation length (d)</td><td>F0</td><td>22.7</td><td>22.4*</td><td>22.6</td><td>22.7</td><td>22.9</td></tr>
<tr><td>F1</td><td>22.3</td><td>22.3</td><td>22.4</td><td>22.8**</td><td>22.9**</td></tr>
<tr><td>F2CT</td><td>23.0</td><td>22.9</td><td>22.5*</td><td>22.9</td><td>23.1</td></tr>
<tr><td>F2R</td><td>22.4</td><td>22.4</td><td>22.7</td><td>22.8</td><td>22.7</td></tr>
<tr><td rowspan="4">Whole litter loss (%)</td><td>F1</td><td>4.3</td><td>0.0</td><td>9.5</td><td>5.0</td><td>4.3</td></tr>
<tr><td>F2</td><td>4.8</td><td>0.0</td><td>8.7</td><td>8.0</td><td>35.0*</td></tr>
<tr><td>F3CT</td><td>0.0</td><td>0.0</td><td>9.1</td><td>9.1</td><td>30.0</td></tr>
<tr><td>F3R</td><td>0.0</td><td>0.0</td><td>0.0</td><td>7.7</td><td>0.0</td></tr>
<tr><td rowspan="4">Live born pups (%)</td><td>F1</td><td>97.1</td><td>96.1</td><td>99.1</td><td>94.9</td><td>96.9</td></tr>
<tr><td>F2</td><td>97.8</td><td>97.4</td><td>97.0</td><td>98.1</td><td>92.2**</td></tr>
<tr><td>F3CT</td><td>94.6</td><td>98.2</td><td>92.2</td><td>89.3</td><td>83.4*</td></tr>
<tr><td>F3R</td><td>97.8</td><td>98.2</td><td>90.0</td><td>84.5*</td><td>95.4</td></tr>
<tr><td rowspan="4">Litter size (no. pups)</td><td>F1</td><td>11.7</td><td>12.4</td><td>10.9</td><td>10.3</td><td>9.2**</td></tr>
<tr><td>F2</td><td>11.8</td><td>9.8</td><td>9.5</td><td>10.0</td><td>7.8**</td></tr>
<tr><td>F3CT</td><td>10.6</td><td>10.8</td><td>8.4</td><td>8.5</td><td>5.5**</td></tr>
<tr><td>F3R</td><td>11.7</td><td>10.5</td><td>9.6</td><td>9.2*</td><td>8.2*</td></tr>
<tr><td rowspan="4">Litter weight (g)
Day 0</td><td>F1</td><td>70.4</td><td>72.2</td><td>65.9</td><td>63.4</td><td>57.1**</td></tr>
<tr><td>F2</td><td>68.7</td><td>57.7</td><td>57.6</td><td>60.8</td><td>46.8**</td></tr>
<tr><td>F3CT</td><td>63.9</td><td>66.5</td><td>50.8</td><td>54.1</td><td>32.4**</td></tr>
<tr><td>F3R</td><td>69.9</td><td>64.3</td><td>58.6</td><td>56.4</td><td>49.9*</td></tr>
<tr><td rowspan="4">Pup survival (to day 22, %)</td><td>F1</td><td>92.4</td><td>89.9</td><td>85.2**</td><td>89.7</td><td>77.6**</td></tr>
<tr><td>F2</td><td>83.9</td><td>88.7</td><td>79.7</td><td>75.8</td><td>48.2**</td></tr>
<tr><td>F3CT</td><td>90.3</td><td>93.0</td><td>80.2</td><td>85.4</td><td>57.6*</td></tr>
<tr><td>F3R</td><td>93.3</td><td>88.7</td><td>86.6</td><td>81.8</td><td>94.4</td></tr>
<tr><td rowspan="3">Preputial separation (day)</td><td>F2</td><td>46.2</td><td>47.5*</td><td>47.7**</td><td>49.0**</td><td>49.1**</td></tr>
<tr><td>F3CT</td><td>44.7</td><td>44.7</td><td>46.9**</td><td>45.8</td><td>45.5</td></tr>
<tr><td>F3R</td><td>45.2</td><td>44.4</td><td>45.1</td><td>44.2</td><td>44.3</td></tr>
</table>
* significantly different to control (p<0.05), ** (p<0.01)</td></tr>
</table>

		Parental toxicity **2.5 ppm (0.3 mg/kg bw/day)** F0, F1 & F2: No significant toxicological effects **10 ppm (1.1 / 1.2 mg/kg bw/day males / females)** F0: No significant toxicological effects F1: *clinical observations:* ↑ cloudy eyes (1/26 males) *ophthalmoscopy*: ↑ corneal opacity (4/20 eyes males), ↑ vascularisation (2/20 males); *microscopic findings in the eye:* ↑ keratitis (5/26 males), corneal vascularisation (6/26 males, cf. 0/26 controls) *macroscopic findings in the kidney:* ↑ pelvic dilatation (18/26 males, cf. 9/26 controls) *microscopic findings in the kidney:* ↑ bilateral hydronephrosis (10/26 males, cf. 0/26 controls) F2: *clinical observations*: ↑ cloudy eyes (1/26 males) F2CT: *macroscopic findings in the kidney:* ↑ pelvic dilatation (8/12 males, cf. 6/12 controls) *microscopic findings in the kidney:* ↑ bilateral hydronephrosis (2/12 males, cf. 0/12 controls) **100 ppm (11.6/12.3 mg/kg bw/day males/females)** F0: *clinical observations* ↑ cloudy eyes (9/26 males, 0/26 females) *ophthalmoscopy*: ↑ corneal opacity/vascularisation (7/20 eyes males, 13/20 eyes females); *microscopic findings in the eye:* ↑ corneal opacity (9/26 females), ↑ hazy eye (7/26 males), ↑ vascularisation (7/26 males, 8/26 females), ↑ keratitis (11/26 males, 8/26) *effects in the liver:* ↑ relative weight (14.6% males, 8.8% females) *effects in the kidney:* ↑ relative weight (11.9% males) F1: *clinical observations:* ↑ cloudy eyes (26/26 males, 13/26 females *microscopic findings in the eye*: ↑ corneal opacity/vascularisation (19/20 eyes males, 16/20 eyes females), ↑ keratitis (25/25 males, 23/26 females); *effects in the kidney:* ↑ relative weight (9.9% males) macroscopic finding: ↑ pelvic dilatation (19/26 males cf. 9/26 controls; 8/26 females cf. 4/26 in controls) microscopic finding: ↑ bilateral hydronephrosis (14/26 males cf. 0/26 controls; 15/26 females, cf. 4/24 in controls) *effects in the liver:* ↑ relative weight (10.6% males, 14.6% females)

		F2: *clinical observations*: ↑ cloudy eyes *microscopic findings in the eyes:* ↑ corneal opacity/vascularisation (16/20 eyes males, ~5/20 eyes females continuous treatment – ghost vascularisation only in 15/20 males recovery, week 27/28) *effects in the kidneys:* ↑ relative weight (17.7% males continuous treatment – 9.2% males recovery) macroscopic finding: ↑ pelvic dilatation (10/12 males cf. 6/12 in controls - continuous treatment. 9/14 males cf. 5/14 males in the recovery group) microscopic finding: ↑ bilateral hydronephrosis (6/12 males cf. 0/12 controls, 2/12 females cf. 0/12 controls, continuous treatment) *effects in the liver:* ↑ relative weight (24% males) **2500 ppm (278 / 306 mg/kg bw/day males / females)** F0: *clinical observations:* ↑ cloudy eyes (16/26 males, 8/26 females) *body weight*: ↓ (3.0% males, 5.1% females week 11) *food utilisation*: ↓ (3.1% males weeks 1 - 4) *microscopic findings in the eye:* ↑ corneal opacity/vascularisation (10/20 eyes males, 19/20 eyes females preterm), ↑ keratitis (18/26 males, 25/26 females) *effects in the kidney:* ↑ relative weight (10.6% males, 4.3% females) *effects in the liver:* ↑ relative weight (16.9% males, 11.2% females) F1: *clinical observations*: ↑ cloudy eyes (25/26 males, 26/26 females) *microscopic findings in the eye*: ↑ corneal opacity/vascularisation (20/20 eyes males, 20/20 eyes females), ↑ keratitis (25/25 males, 25/25 females at term) *effects in the kidney:* ↑ relative weight (11.4% males) macroscopic finding: ↑ pelvic dilatation (21/26 males cf. 9/26 controls; 12/26 females cf. 4/26 controls) microscopic finding: ↑bilateral hydronephrosis (21/26 males cf. 0/26 controls; 14/26 females cf. 4/24 controls) *effects in the liver:* ↑ weight (10.6% males) F2: *clinical observations*: ↑ cloudy eyes (26/26 males, 24/26 females); *microscopic findings in the eyes:* ↑ corneal opacity/vascularisation (20/20 eyes males, 18/20 eyes females continuous treatment – ghost vascularisation only in 14/20 males, 15/20 females recovery, week 27/28) *effects in the kidneys:* ↑ relative weight (26.4% males, continuous treatment – 10.3% males recovery) macroscopic finding: ↑ pelvic dilatation (continuous treatment: 12/12 males cf. 6/12 in controls; 5/12 females cf. 1/12 in controls. Recovery: 14/14 males cf. 5/14 in controls; 6/14 females cf. 0/13 in controls) microscopic finding: ↑bilateral hydronephrosis (continuous treatment: 8/12 males cf. 0/12 controls; 1/12 females cf. 0/12 controls. Recovery: 5/14 males cf. 2/14 controls; 5/14 females, cf. 0/13 controls) *effects in the liver:* ↑ relative weight (27% males) #Parental NOAEL = 2.5 ppm (0.3 mg/kg bw/day) based on increased organ weights at ≥ 10 ppm.

		Offspring toxicity **2.5 ppm (0.3 mg/kg bw/day)** F1, F3: No significant toxicological effects F2: microscopic finding in the kidney: ↑ hydronephrosis (4/9 males, cf. 1/10 controls) **10 ppm (1.1 / 1.2 mg/kg bw/day males / females)** F1: *effects in the kidney:* macroscopic finding: ↑ hydronephrosis (6/39 males cf. 2/44 controls; 7/42 females cf. 2/49 controls) microscopic finding: ↑ hydronephrosis (2/5 males cf. 1/5 controls; 2/5 females cf. 1/5 controls) F2: *effects in the kidney:* macroscopic finding: ↑ hydronephrosis (8/39 males cf. 5/45 controls; 5/37 females cf. 5/45 controls) microscopic finding: ↑ hydronephrosis (4/10 males cf. 1/10 controls; 4/10 females cf. 3/10 controls) F3CT: *ophthalmoscopy*: ↑ corneal opacity/vascularisation (2/5 males) **100 ppm (11.6 / 12.3 mg/kg bw/day males / females)** F1: *Clinical observations:* ↑ cloudy eyes (1 pup/1 litter) *microscopic effects in the eye:* ↑ keratitis (4/5 males, 4/5 females full pm) +/corneal vascularisation *kidney effects:* macroscopic finding: ↑ hydronephrosis (15/38 males cf. 2/44 controls; 17/43 females cf. 2/49 controls) microscopic finding: ↑hydronephrosis (2/5 males cf. 1/5 controls; 3/5 females cf. 1/5 controls) F2: *Clinical observations*: ↑ cloudy eyes (48 pups/9 litters) *microscopic effects in the eye:* ↑ keratitis (9/10 males, 5/10 females) *effects in the kidney:* macroscopic finding: ↑hydronephrosis (20/50 males, cf. 5/45 controls; 11/37 females, cf. 5/45 controls) microscopic finding: ↑ hydronephrosis (7/10 males cf. 1/10 controls; 7/10 females cf. 3/10 controls),interstitial nephritis (5/10 females) F3CT: *Clinical observations*: ↑ cloudy eyes (3 pups/3 litters) *ophthalmoscopy*: ↑ corneal opacity/vascularisation (7/7 males, 8/10 females) *effects in the kidney:* macroscopic findings: ↑ hydronephrosis (7/12 males cf. 2/22 controls; 15/34 females cf. 1/33 controls), ↑ bilateral pelvic dilatation (5/33 males cf. 0/50 controls; 9/73 females cf. 1/57 controls)

		2500 ppm (278/306 mg/kg bw/day males/females) F1: *clinical observations:* ↑ cloudy eyes (65 pups/14 litters) *microscopic effects in the eye:* ↑ keratitis (5/5 males, 5/5 females) *Effects in the kidney:* ↑ kidney weight (11.4% males, 14.7% females) macroscopic finding: ↑hydronephrosis (16/54 males cf. 2/44 controls; 8/42 females cf. 2/49 in controls) microscopic finding: ↑ hydronephrosis (3/5 males cf. 1/5 controls; 3/5 females cf. 1/5 controls) F2: *clinical observations:* ↑ cloudy eyes (54 pups/11 litters) *Microscopic findings in the eye:* ↑ keratitis (9/10 males, 8/9 females); *Findings in the kidney:* macroscopic finding: ↑ hydronephrosis (10/13 males cf. 5/45 controls; 10/10 females cf. 5/45 controls) microscopic finding: ↑ hydronephrosis (9/10 males cf. 1/10 controls; 9/9 females cf. 3/10 controls), interstitial nephritis (7/9 females) *Effects in the liver:* ↑ liver weight (10.1% females) F3CT: *clinical observations*: ↑ cloudy eyes (17 pups/5 litters) *ophthalmoscopy*: ↑ corneal opacity/vascularisation (3/4 males, 6/7 females) effects in the kidney: macroscopic findings: ↑ bilateral hydronephrosis (5/8 males cf. 2/22 controls; 9/10 females cf. 1/33 controls); bilateral pelvic dilatation (3/17 males cf. 0/50 controls; 5/59 females cf. 1/57 controls) *#NOAEL not identified due to increased incidences of hydronephrosis in male F2 pups at all dose levels.*

Single generation reproductive toxicity study in the rat (non-guideline)

GLP

Alpk:APfSD Wistar rats, 20 females per group

Mesotrione (purity: 96.8%)

Anonymous (1997q) + (2000b)

RAR: B.6.8.2.8

Dose levels: 0 or 2500 ppm mesotrione (nominal in diet) co administered with 0, 0.5, 1, or 2 % tyrosine

Dose groups:

Grp #	Dietary concentration	
	Meso (ppm)	Tyr (% w/w)
1	0	0
2		0.5
3		1.0
4		2.0
5	2500	0
6		0.5
7		1.0
8		2.0

Meso = mesotrione

Tyre = tyrosine

Group 8

Animals terminated on days 8-11 due to adverse clinical signs

↓ body weight (13%)

Observation		Sex	Group number			
			1	2	3	4
Litter size		--	12.05	11.95	11.57	12.39
Day 1	Live	m/f	11.79	11.37	11.07	11.39
		M	7.05	5.89	5.79	6.00
		F	4.74	5.47	5.29	5.39
	Dead	m/f	0.26	0.58	0.50	1.00
		M	0.00	0.26	0.21	0.56
		F	0.26	0.32	0.29	0.44
Day 5	Live	m/f	11.11	10.67	10.67	10.88
		M	6.74	5.56	5.50	5.94
		F	4.37	5.11	5.17	4.94
	Post-natal deaths (%)	m/f	6.91	7.31	2.97	8.68
		M	4.35	6.50	4.86	7.00
		F	12.51	7.17	2.38	9.90
Total litter loss			0/19	1/19	2/14	1/17
Plasma tyrosine (µM)			182.2	199.5	209.4	293.3

Observation		Sex	Group number			
			5	6	7	8
Litter size		--	11.78	12.12	11.28	
Day 1	Live	m/f	11.06	11.18	9.89	
		M	5.94	5.35*	4.83	
		F	5.11	5.82	5.06	
	Dead	m/f	0.72	0.94	1.39*	
		M	0.33	0.35*	0.67*	
		F	0.39	0.59	0.67	
Day 5	Live	m/f	9.67	8.54*	5.20**	
		M	5.39	3.77**	2.60**	
		F	4.28	4.77	2.60*	
	Post-natal Deaths (%)	m/f	14.50	22.47*	43.16**	
		M	10.30	20.53*	45.50**	
		F	26.38	22.56	36.53*	
Total litter loss			0/18	4/17	8/18	
Plasma tyrosine (µM)			2051	2643	2010	

Mouse (CD-1) Two generation reproductive toxicity study, OECD 416 GLP 26/sex/group Purity: 96.8% Exposure: Continuous in the diet Anonymous (1997r) RAR: B.6.6.1.2	0, 10, 50, 350, 1500 and 7000 ppm (in diet), equivalent to: 0, 2.1, 10, 71, 312 and 1472 mg/kg bw/d in F0 males; 0, 2.1, 10, 71, 302 and 1430 mg/kg bw/d in F1 males; 0, 2.4, 12, 84, 372 and 1632 mg/kg bw/d in pre-mate F0 females; 0, 2.1, 10, 73, 300 and 1430 mg/kg bw/d in F0 gestating females; 0, 13, 70.1, 482, 2001 and 8726 mg/kg bw/d in lactating F0 females; 0, 2.4, 11.4, 81, 354 and 1673 mg/kg bw/d in pre-mate F1 females; 0, 2.0, 9.8, 74, 303 and 1491 mg/kg bw/d in gestating F1 females and 0, 66, 422.4, 1879 and 8260 mg/kg bw/d in lactating F1 females.	***Reproductive toxicity*** No effects at any dose level. NOAEL 7000 ppm (1430 mg/kg bw/day). ***Parental toxicity*** **10 ppm (2 mg/kg bw/day)** F0: no significant toxicological effects F1: no significant toxicological effects. Approximate 3.7/2.6 fold increase in plasma tyrosine for males/females compared with controls. **50 ppm (10 mg/kg bw/day)** F0: no significant toxicological effects F1: Approximate 4.7/3.0 fold increase in plasma tyrosine for males/females compared with controls. **350 ppm (71 mg/kg bw/day)** F0: no significant toxicological effects F1: Approximate 5.9/5.1 fold increase in plasma tyrosine for males/females compared with controls. **1500 ppm (300 mg/kg bw/day)** F0: no significant toxicological effects F1:↓ food consumption during lactation (11.8% week 3); Approximate 6.1/5.7 fold increase in plasma tyrosine for males/females compared with controls **7000 ppm (1430 mg/kg bw/day)** F0:↓ body weight during lactation (13.1% day 15); ↓ food consumption during lactation (15.1% week 3); ↑ cataractous change in the eye 3/26 males, 0/26 controls) F1: ↑ opaque eye (4/26 males, 6/26 females – 0 incidence in controls); ↓ body weight during lactation (11.5% day 15); ↓ food consumption during lactation (21.9% week 3); ↑ liver weight adjusted for body weight (10.4% males, 9.1% females); ↑ kidney weight adjusted for body weight (17.4% males, 8.8% females); cataractous change in the eye 8/26 males, 6/26 females (0 incidence in controls). Approximate 6.9/6.4 fold increase in plasma tyrosine for males/females compared with controls. #NOAEL 10 ppm (2 mg/kg bw/day) – based on organ weight changes. ***Offspring toxicity*** **10 ppm (2 mg/kg bw/day)** F1: ↓ body weight (7.5% males, 5.8% females) F2: No effects. Approximate 9/8 fold increase in plasma tyrosine for males/females compared with controls. **50 ppm (10 mg/kg bw/day)** F1: ↓ body weight (5.7% males, 5% females) F2: No effects. Approximate 19/12 fold increase in plasma tyrosine for males/females compared with controls.

350 ppm (71 mg/kg bw/day)

F1: ↓ body weight (7.5% males, 6.2% females)

F2: No effects. Approximate 32/22 fold increase in plasma tyrosine for males/females compared with controls.

1500 ppm (300 mg/kg bw/day)

F1: ↓ body weight (10.9% males, 9.2% females day 29)

F2: ↓ body weight (8.0% males, 5.6% females day 29); ↑ opaque/cloudy eyes 3/26 males, 3/35 females; cataractous change in 2/16 males. Approximate 31/24 fold increase in plasma tyrosine for males/females compared with controls.

7000 ppm (1430 mg/kg bw/day)

F1: ↓ body weight (19.2% males, 16.5% females day 29); ↑ liver weight adjusted for body weight (14% males, 12% females); cataractous change in the eye 4/10 males, 2/10 females – 0 incidence in controls. Delay in preputial separation by 2 days.

F2: ↓ body weight (16.7% males, 11.6% females day 29); ↑ liver weight adjusted for body weight (11.8% males, 8.5% females); ↑ opaque/cloudy eyes 10/33 males, 3/31 females; cataractous change in the eye 11/18 males, 3/12 females(0 incidence in controls). Delay in preputial separation by 3 days. Approximate 51/40 fold increase in plasma tyrosine for males/females compared with controls.

#NOAEL 350 ppm (71 mg/kg bw/day)

Plasma tyrosine levels:

Group		**Terminal plasma tyrosine concentration (µM)**					
		0	**10**	**50**	**350**	**1500**	**7000**
F1 Adults	**M**	149	551	704	874	906	1023
	F	124	322	369	634	710	798
F2 Pups	**M**	27	237	500	847	819	1354
	F	33	254	415	735	802	1343

4.11.1.1 Non-human information

Guideline rat multigeneration study in Alpk:AP$_f$SD rats

Rats (26/sex/dose level) were fed diet containing 0, 2.5, 10, 100 or 2500 ppm mesotrione (equivalent to ~ 0, 0.3, 1.1, 11.6 and 278 mg/kg bw/d in males and ~0, 0.3, 1.2, 12.4 and 307 mg/kg bw/d in females). F2 animals were fed experimental diets until Week 14, after which approximately half of the animals continued with the same treatment (F2CT) while the remainder were assigned to a recovery sub-group (F2R) and fed control diet. At Week 18, F2 sub-groups were mated to produce F3 litters (F3CT and F3R).

Whole litter loss was significantly increased and the percentage of pups born alive was significantly decreased in the F2 and F3CT litters at the top dose (2500ppm). No effect was seen in F1 or F3R litters.

Litter size was significantly reduced in all generations at the top dose; the magnitude of the effect was greatest in the F3CT animals. Pup survival (to day 22) was also significantly decreased at this dose in F1, F2 and F3CT litters. There was no difference between the survival of male and female pups.

Dose-related decreases in litter weight were observed in the F1, F2, F3CT and F3R generations. Values were statistically significant at the top dose. Pup weights were not significantly affected by treatment.

Effects in the adults

Cloudy eyes were noted as follows; in adults of both sexes and all generations at the top dose (2500ppm), in male adults of all generations and F0/F1 females at 100 ppm and in F1 and F2R males at ~1 mg/kg bw/d. Slight (<10%) reductions in body weight were noted in adults of each generation at the top dose, and in F1/F2 males at ≥11 mg/kg bw/d.

The target organs were the eyes and kidney. In the eyes, increased incidences of corneal opacity and corneal vascularisation were apparent in both sexes of F0 adults at ≥11.6 mg/kg bw/d. Findings were more severe in males. Similar effects were seen in F1 males at ≥1.1 mg/kg bw/d and in F1 females at ≥12.3 mg/kg bw/d. Prior to the division of F2 adults into sub-groups at Week 13, corneal opacity and vascularisation were seen in both sexes at ≥11.6 mg/kg bw/d and in one male at 1.1 mg/kg bw/d. Cataracts were also noted in F2 males at ≥11.6 mg/kg bw/d. Findings persisted in F2 animals assigned to the continuous treatment sub-group. Evidence of recovery was seen at ≥11.3 mg/kg bw/d in the F2 recovery sub-group at Week 18. No ocular findings were noted in the F3 recovery sub-group.

Increased mean relative kidney weights were noted in treated adults (all generations) at ≥1.1 mg/kg bw/d (up to 26.4% in F1 and F2CT adults at the top dose). Some evidence for the reversibility of kidney weight increases was seen in F2R animals. Pelvic dilatation (macroscopic finding) was increased in F1 and F2CT males at ≥ 1.1 mg/kg bw/d, F1 and F2CT females at ≥ 12.4 mg/kg bw/d, F2R males at 0.3 mg/kg bw/d and F2R females at 1.2 mg/kg bw/d. Bilateral hydronephrosis (microscopic finding) was increased in F1 and F2CT males at ≥ 1.1 mg/kg bw/d, in F2R males at ≥11.6 mg/kg bw/d, and in F1, F2CT and F2R females at ≥ 12.4 mg/kg bw/d. F0 adults (who were not exposed until approximately 4 weeks of age) were not affected.

Mean testes weights were slightly (but significantly) increased at ≥1.1 mg/kg bw/d in F0 males only (↑4.7%, 4.2% and 4.5% at 1.1, 11.6 and 278 mg/kg bw/d). Due to a lack of dose response, and the absence of this effect in the F1 and F2 animals, the slight increase in testes weight is not considered to be the result of treatment with mesotrione. Mean epididymal weights were slightly (but significantly) lower in F1 males at ≥11.6 mg/kg bw/d (↓7.0% and 8.4% at 11.6 and 278 mg/kg bw/d). As this effect was not seen in the F0 or F2 animals, it is not considered to be the result of treatment with mesotrione.

Effects in the offspring

The target organs in the pups were the eyes and kidneys.

Ocular opacity was noted in both sexes of F1 pups at the top dose, and in both sexes of F2 pups at ≥11.6/12.4 mg/kg bw/d. Microscopic findings consisted of corneal keratitis and vascularisation, which were present in both sexes of F1 and F2 pups at ≥11.6/12.4 mg/kg bw/d. Corneal opacity and vascularisation were seen in F3 pups of the continuous treatment sub-group at ≥2.5 ppm in males and ≥100 ppm in females. Iridic effects were also observed in high dose animals of all generations.

Mean relative kidney weights were increased in both sexes of F2 pups at the top dose (↑11.4% in males, ↑14.7% in females). The incidence of hydronephrosis (macroscopic finding) was increased in F1 and F2 pups at ≥ 1.1/1.2 mg/kg bw/d, and in F3CT pups at ≥11.6/12.4 mg/kg bw/d. Dose-related increases in the incidence of hydronephrosis (microscopic finding) were observed in F1 and F2 pups.

Non-guideline single generation study in Alpk:AP$_f$SD rats

Pregnant female rats (20/dose group) were fed diet containing 2500ppm mesotrione (96.8% purity) and/or varying concentrations of tyrosine (see Table 17 for details). The study was designed to evaluate the role of tyrosine in the mesotrione induced reproductive effects of reduced litter size, decreased pup survival and bilateral hydronephrosis. Bodyweights were evaluated on Days 1, 4, 7, 15 and 22 of gestation and on Days 1 and 5 of lactation. Blood samples for the determination of plasma tyrosine levels were collected from 3 animals/group after 48 hours.

Plasma tyrosine concentrations were slightly increased in groups receiving tyrosine alone (182.2, 199.5, 209.4 and 293.3 µM in groups receiving 0, 0.5, 1.0 and 2% (w/w) tyrosine in the diet) and markedly increased in groups receiving mesotrione (2061, 2643 and 2010 µM in groups receiving 2500ppm with 0, 0.5 and 1% (w/w) tyrosine respectively). However, it is noted that in the groups receiving mesotrione, there was no dose-response relationship between the level of dietary tyrosine administered and the resulting levels of plasma tyrosine.

Mean bodyweights of animals receiving 2500 ppm mesotrione + 2% tyrosine were significantly lower than controls at Day 4 (↓11.4%) and Day 7 (↓13%). Animals in this group were terminated on Days 8-11 of the study due to adverse clinical signs and no further data is available on them.

Ocular opacity was noted in groups administered both mesotrione and tyrosine. Increased incidences of hunched posture, piloerection and staining of the coat with urine were also noted in these animals.

In the group receiving 2500 ppm mesotrione and 1% (w/w/) tyrosine, mean litter size was slightly (but not significantly) lower than controls (11.28, cf. 12.05 in controls). The number of dead pups in this group was slightly (but significantly) increased at Day 1 *post partum*. Pup survival at Day 5 *post partum* was decreased in all mesotrione treated groups; survival decreased with increasing dietary tyrosine concentration. Total litter loss was also increased in the groups receiving 2500 ppm mesotrione and 0.5% (w/w) tyrosine and 2500 ppm mesotrione and 1% (w/w) tyrosine. The incidences were 4/18 and 8/18 respectively compared to 0/19 in controls. No such effects were observed in the groups receiving mesotrione only or tyrosine only.

Guideline multigeneration study in Alpk:AP$_f$CD-1 mice

Mice (26/sex/dose level) were administered diet containing 0, 10, 50, 350, 1500 or 7000 ppm mesotrione (96.8% purity), equivalent to approximately 0, 2.1, 10, 71, 312 and 1472 mg/kg bw/d in males, 0, 2.4, 12, 84, 372 and 1632 mg/kg bw/d in non-lactating females and 0, 13, 70.1, 482, 2001 and 8726 mg/kg bw/d in lactating females.

There were no treatment related effects on reproductive parameters.

Effects in the adults

In F0 females, bodyweights and food consumption were significantly reduced from day 5-15 of lactation at 7000 ppm (body weight ↓13% at day 15). Bodyweights of F1 males at 7000 ppm were significantly lower than controls at weaning and throughout the pre-mating period (↓12.3% at week 1 of the pre-mating period). Food consumption was reduced during the pre-mating period in these

animals. Bodyweights of 7000 ppm F1 females were comparable to controls at day 15 of gestation, but were lower throughout lactation (up to 17.3%). This was associated with reduced food consumption.). No significant effects on bodyweight or food consumption were seen in other dose groups.

The target organs in adults were the eyes. Ocular effects (cloudy, opaque or discharging eyes) were noted with slightly increased frequency in F0 males at the top dose. Single incidences were also seen at lower dose levels, however no clear dose-response relationship was apparent. A single incidence of cloudy eyes was seen in F0 females at the top dose. Similar macroscopic findings were seen in F1 males and females. Microscopically, increased incidences of unilateral or bilateral cataractous change were seen in F0 males and F1 animals of both sexes at the top dose. Single incidences of retinal detachment were seen in both sexes at the top dose; in the RAR, these findings were considered to be secondary to the lenticular effects seen in the affected animals.

Effects in the pups
Significant (i.e., >10%) reductions in body weight were observed in F1 pups at ≥1500 ppm and F2 pups at the top dose only. Preputial separation time was slightly (but significantly) delayed in F1 males at 1500 and 7000 ppm, and in F2 pups at the top dose only.

The target organs in the pups were the eyes. Ocular discharge was apparent in F1 and F2 pups of all treated groups from day 15 (late lactation). No clear dose-response was apparent; however no incidences were seen in control pups. Ocular opacity was also noted in six F2 pups at day 29 (all affected pups were from the same litter). Opaque or cloudy eyes were observed with slightly increased incidence in 7000 ppm male F1 pups subjected to full necropsy at Day 29. No such increase was apparent in F1 females. Microscopically, bilateral or unilateral cataractous change was apparent with increased incidence in both sexes at 7000 ppm. Single incidences of retinal detachment were seen at 7000 ppm in F1 males and females; the RAR states that these findings are considered to be secondary to the lenticular effects seen in the affected animals.

Mean liver weights of F1 pups were significantly increased at 350 ppm (females only, ↑10.5%) and 7000 ppm (both sexes, ↑14.0% and 12.0% in males and females, respectively). Liver weights of F2 males were significantly increased at 7000 ppm (↑11.8%); no effect was seen in females). Testes weight was increased in all groups of treated F2 males. Values were statistically significant at 50 and 1500 ppm, however there was no clear dose-response relationship (↑14.5%, 21.7%, 14.5%, 30.1%, 12.0% at 10, 50, 350, 1500 and 7000 ppm).

4.11.1.2 Human information

No data are available.

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

Developmental toxicity of mesotrione has been investigated in rats, mice and rabbits. The following studies are available: rats - guideline developmental toxicity study; mice - non-guideline preliminary developmental toxicity study and a guideline developmental toxicity study; rabbits - guideline developmental toxicity study and a non-guideline study investigating the effects mesotrione and tyrosine on development. These studies are summarised in Table 18. Further information, based on the RAR, is provided below the table.

Table 18: Summary table of relevant reproductive toxicity studies – Development

↑/↓ = increased/decreased compared to control. Unless otherwise stated, effects were seen in both sexes.

*significantly different to controls ($p<0.05$), ** $p<0.01$

#The values for the NOAELs are provided for information only. All NOAEL values provided in this CLH Report have been taken from the Renewal Assessment Report (RAR), produced in accordance with Directive 91/414/EEC.

<table>
<tr><th>Method</th><th>Dose levels</th><th>Observations and remarks
(effects of major toxicological significance)</th></tr>
<tr><td colspan="3">Rats</td></tr>
<tr><td>Developmental toxicity study in rats

OECD 414

GLP compliant

Alpk:AP_fSD rats (mated females – 24/dose level)

Mesotrione (96.8% purity) in deionized water

Anonymous (1997s)

RAR: B.6.6.2.1</td><td>0, 100, 300 or 1000 mg/kg bw/d</td><td><u>Fetal findings:</u>
<table>
<tr><th colspan="2" rowspan="2">Finding</th><th colspan="4">% Fetal Incidence (Litter incidence)</th><th rowspan="2">Historical Range</th></tr>
<tr><th>0</th><th>100</th><th>300</th><th>1000</th></tr>
<tr><td colspan="2">Minor skeletal defects</td><td>31.7</td><td>48.1*</td><td>56.7**</td><td>74.6**</td><td>---</td></tr>
<tr><td colspan="2">Skeletal variants</td><td>71.5</td><td>84.1**</td><td>88.4**</td><td>97.3**</td><td>---</td></tr>
<tr><td colspan="2">Odontoid not ossified</td><td>19.5
(95.8)</td><td>40.9**
(100)</td><td>48.4**
(95.8)</td><td>67.3**
(100)</td><td>8.3-36.3</td></tr>
<tr><td rowspan="6">Cervical centra not ossified</td><td>2nd</td><td>22.2
(87.5)</td><td>59.4**
(100)</td><td>69.9**
(95.8)</td><td>86.7**
(100)</td><td>15.6-36.3</td></tr>
<tr><td>3rd</td><td>3.6
(29.2)</td><td>19.1**
(66.7*)</td><td>30.1**
(70.8**)</td><td>58.3**
(100**)</td><td>2.5-8.0</td></tr>
<tr><td>4th</td><td>2.4
(29.2)</td><td>8.1**
(54.2)</td><td>14.1**
(54.2)</td><td>34.3**
(83.3**)</td><td>1.3-3.4</td></tr>
<tr><td>5th</td><td>0.3
(4.2)</td><td>2.2
(25.0)</td><td>6.7**
(41.7**)</td><td>15.1**
(62.5**)</td><td>0.3-1.9
---</td></tr>
<tr><td>6th</td><td>0.9
(12.5)</td><td>0.6
(4.2)</td><td>2.6
(20.8)</td><td>6.2**
(45.8)*</td><td>0.0-1.3
---</td></tr>
<tr><td>7th</td><td>0.0
(0.0)</td><td>0.0
(0.0)</td><td>0.3
(4.2)</td><td>1.2
(12.5)</td><td>---
---</td></tr>
<tr><td colspan="2">7th Transverse process partially ossified</td><td>21.9
(87.5)</td><td>9.4**
(66.7)</td><td>4.5**
(41.7**)</td><td>12.7**
(70.8)</td><td>14.2-30.2
---</td></tr>
<tr><td colspan="2">5th Sternebra partially ossified</td><td>28.0
(91.7)</td><td>10.9
(70.8)</td><td>24.4
(87.5)</td><td>36.4*
(91.7)</td><td>18.2-28.0
---</td></tr>
<tr><td colspan="2">Extra short 14th rib</td><td>3.0
(29.2)</td><td>23.8**
(83.3**)</td><td>20.5**
(70.8**)</td><td>19.4**
(62.5*)</td><td>1.8-6.9
---</td></tr>
<tr><td colspan="2">Calcaneum not ossified</td><td>25.5
(87.5)</td><td>50.6**
(100)</td><td>71.2**
(95.8)</td><td>89.8**
(100)</td><td>25.5-95.0
---</td></tr>
<tr><td colspan="2">Mean litter manus score #</td><td>3.82</td><td>3.97*</td><td>4.03**</td><td>4.13**</td><td>3.27-3.96</td></tr>
<tr><td colspan="2">Mean litter pes score #</td><td>3.98</td><td>4.20**</td><td>4.23**</td><td>4.42**</td><td>3.98-4.17</td></tr>
</table>
#Maternal NOAEL not identified due to decreased bodyweight gain and food consumption at all dose levels. A fetal NOAEL not identified due to the increased incidence of minor skeletal defects and skeletal variants in all dose groups.</td></tr>
</table>

Mice

Preliminary developmental toxicity study in the mouse

Non-guideline

GLP compliant

Crl:CD-1(ICR)BR mice (mated females – 14/dose)

Mesotrione (96.8% purity) in deionised water

Anonymous (1998)

RAR B.6.6.2.3

0, 300 or 600 mg/kg bw/d

300 mg/kg bw/d

↑ number of live fetuses (13.2, cf. 10.1 in controls)

↑ mean litter weight (18.6g, cf. 15.7g in controls)

600 mg/kg bw/d

↑ number of live fetuses (11.9, cf. 10.1 in controls)

↑ mean litter weight (17.7g, cf. 15.7g in controls)

Minor skeletal effects:

	Dose Group (mg/kg bw/d)		
	0	300	600
Minor skeletal abnormalities	14.2%	9.3%	22.9%
Incomplete ossification occipita	15.6	14.9	26.5
4th cervical centra incomplete ossification	7.6	4.8	12.0
5th cervical centra incomplete ossification	3.5	2.4	4.5
14 thoracic vertebrae	20.7	25.2	28.5
5 lumbar vertebrae	20.7	24.3	28.5
Astragalus not ossified	1.4	0.0	6.0

#Maternal NOAEL = 600 mg/kg bw/d. Fetal NOAEL = 300 mg/kg bw/d

Developmental toxicity study in mice

OECD 414

GLP compliant

Alpk:AP$_f$CD-1 mice (mated females, 30/dose level)

Mesotrione (96.8% purity) in distilled water

Anonymous (1999a)

0, 0, 10, 60, 150 or 600 mg/kg bw/d

Fetal Findings:

Observation		Dose Level (mg/kg bw/d)					
		0	0	10	60	150	600
Mean litter weight (g)		15.7	15.6	16.6	16.5	16.5	17.1
Mean fetal weight (g)		1.25	1.29	1.34*	1.32	1.32	1.34*
External/visceral findings							
Gastroschisis		0.0	0.0	0.0	0.0	0.3	0.7
Hind limb malrotated		0.0	0.0	0.0	0.0	0.0	0.7
Skeletal findings [fetal incidence (litter incidence)]							
Supra-occipital: incomplete ossification	slight	0.7 (8.3)	1.9 (23.1)	7.3** (37.5)	6.3** (36.0)	6.1** (40.0*)	3.4 (34.8)

RAR B.6.6.2.3		Cervical centra not ossified	2nd	3.0 (29.2)	1.6 (11.5)	4.3 (20.8)	6.7** (28.0)	3.5 (32.0)	6.1** (34.8)
			3rd	13.0 (54.2)	3.5 (26.9)	11.9 (50.0)	11.4 (56.0)	12.5* (52.0)	17.0** (65.2)
			4th	15.1 (54.2)	5.4 (26.9)	14.6 (58.3)	12.1 (52.0)	14.7 (56.0)	21.1** (73.9*)
			5th	12.4 (54.2)	3.5 (26.9)	11.9 (54.2)	11.1 (52.0)	12.8 (48.0)	19.7** (73.9*)
			6th	7.4 (29.2)	2.5 (23.1)	9.6* (41.7)	8.3 (44.0)	6.1 (40.0)	10.2** (52.2)
			7th	1.3 (4.2)	0.6 (7.7)	5.3** (29.2)	5.7** (20.0)	0.6 (8.0)	5.1** (30.4)
		Odontoid not ossified		2.7 (25.0)	2.5 (23.1)	5.0 (29.2)	6.7** (28.0)	4.5 (32.0)	7.5** (47.8)
		2nd cervical arch narrowed		5.7 (41.7)	4.4 (34.6)	2.3 (25.0)	2.2 (28.0)	1.9* (16.0)	1.0** (8.7*)
		Sternebra 6 incompletely fused		7.4 (41.7)	3.5 (23.1)	9.9* (45.8)	7.3 (32.0)	6.7 (44.0)	15.0** (69.6**)
		Sternebra 6 incompletely ossified		2.3 (16.7)	0.3 (3.8)	3.6* (29.2)	5.1** (12.0)	3.2 (28.0)	4.8** (34.8*)
		Short rib 14		1.0 (8.3)	1.0 (11.5)	3.0 (8.3)	3.2* (16.0)	1.6 (12.0)	5.4** (30.4)
		Long rib 7		5.7 (33.3)	5.1 (34.6)	8.3 (37.5)	3.2 (28.0)	3.2 (32.0)	2.0* (21.7)
		Calcaneum ossified		28.8 (58.3)	36.2 (84.6)	31.1 (62.5)	18.4** (60.0)	22.0** (64.0)	11.2** (47.8)

#NOAEL (maternal) = 600 mg/kg bw/d. NOAEL (fetal) = 150 mg/kg bw/d based on skeletal findings at 600 mg/kg bw/d

Rabbit

Developmental toxicity study in the rabbit

OECD 414

GLP compliant

New Zealand White rabbits (mated females, 20/dose level)

Mesotrione (96.8% purity) in deionized water

Anonymous (1997t)

RAR B.6.6.2.2.

0, 100, 250 or 500 mg/kg bw/d

Fetal findings

Parameter	% fetal incidence (litter incidence)				Historical range
	0	100	250	500	
Odontoid partial ossification	40.0 (72.2)	64.5** (100)	61.7** (100)	72.0** (100)	26.3-45.7 ---
7th Cervical transverse process full ossification	3.3 (11.1)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	--- ---
7th Cervical transverse process partial ossification	6.7 (22.2)	0.8* (7.1)	0.7* (5.9)	0.0** (0.0)	0-6.7 ---
7th Cervical rib(s)	4.0 (16.7)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	--- ---
3rd Lumbar transverse process ossified	8.0 (22.2)	0.8** (7.1)	1.3* (11.8)	2.5 (18.8)	2.9-13.8 ---
27 Pre-sacral vertebrae	28. 0 (66.7)	58.9** (100*)	65.1** (88.2)	69.5** (93.8)	14.6-36.5 ---
5th Sternebra partial ossification	52.0 (94.4)	32.3** (92.9)	28.9** (76.5)	24.6** (62.5)	13.3-52.0 ---
Extra (13th) ribs (short/floating)	14.0 (61.1)	5.6* (35.7)	2.7** (17.6*)	5.9* (37.5)	4.3-14.0 ---
Extra (13th) ribs (normal length)	42.0 (83.3)	78.2** (100)	82.6** (100)	81.4** (100)	17.1-55.2 ---
Manus score of 5	0.0	2.4	5.4**	4.2*	---
Pes score of 1	97.3	92.7	92.6	88.1**	---
Pes score of 2	2.7	7.3	7.4	11.9**	---
Mean litter manus score #	2.83	2.97	2.87	2.94	2.78-3.14
Mean litter pes score #	1.02	1.08	1.06	1.11	1.02-1.11

#NOAEL (maternal) = 100 mg/kg bw/d. A fetal NOAEL was not identified due to skeletal findings in all treated groups.

Investigation of the effects of mesotrione and tyrosine on developmental toxicity in the rabbit Non-guideline GLP compliant New Zealand White rabbits (mated females, 20/dose group) Mesotrione (96.8% purity) and/or tyrosine in the diet Anonymous (2000c) RAR B.6.6.2.2	Group I: control Group II: 1% tyrosine Group III: mesotrione (500 mg/kg bw/d) Group IV: 1% tyrosine + 500 mg/kg bw/d mesotrione	

Parameter		Dose Group			
		I	II	III	IV
		Control	Tyrosine	Mesotrione	Tyrosine + mesotrione
Plasma tyrosine	Day 8 (pre)	84	75	81	79
	Day 8 (+12h)	69	199**	560**	1202**
	Day 8 (+24h)	77	90	177**	414**
	Day 14 (pre)	83	137**	246**	363**
	Day 14 (+12h)	63	171**	528**	1077**
	Day 14 (+24h)	70	103**	179**	269**
	Day 20 (pre)	57	112**	171**	338**
	Day 20 (+12h)	51	136**	464**	955**
	Day 20 (+24h)	53	95**	166**	331**
	Day 29	53	57	67**	66**
HPPD (µl/min/mg)	Liver	1.90	1.94	0.81**	0.87**
	Kidney	0.55	0.51	0.14**	0.17**
TAT (nmol/min/mg)	Liver	3.82	3.899	3.77	3.72
	Kidney	0.81	0.66	0.34**	0.47**
Fetal parameters					
Aorta dilated	Extreme	-	0.8 (7.1)	1.4 (11.1)	2.1 (11.8)
	Slight	0.7 (6.3)	0.8 (7.1)	2.0 (11.1)	-
Pulmonary artery constricted	Extreme	-	-	0.7 (5.6)	1.4 (11.8)
	Slight	-	-	0.7(5.6)	-
Extra vessel from aortic arch		-	0.8 (7.1)	4.8* (27.8*)	6.9** (29.4*)
Skeletal variants		66.7 (100)	63.9(100)	89.7** (100)	98.4** (100)
Holes in parietal		-	3.3* (7.1)	1.4 (11.1)	2.1 (17.6)

		Odontoid ↓ ossification		-	0.8(7.1)	13.6** (38.9**)	14.5** (52.9**)
		Odontoid not ossified		-	-	2.7 (11.1)	1.4 (11.8)
		Odontoid displaced		-	-	-	2.8 (23.6)
		Lumbar arch process lengthened		9.2 (37.5)	4.1 (21.4)	2.7* (16.7)	0.0* (0.0*)
		Lumbar arch 6th transverse process not ossified		0.7 (6.3)	1.6 (14.3)	4.1 (16.7)	2.8 (23.5)
		Sternebra 5 ↓ossification		22.5 (81.3)	11.5* (57.1)	11.6* (44.4*)	5.5** (23.5**)
		Sternebra 5 not ossified		16.9 (81.3)	6.6* (28.6*)	10.2 (44.4*)	6.2** (35.3*)
		Rib 13 long		21.8 (68.8)	35.2* (92.9)	73.5** (88.9)	92.4** (100*)
		Pubis ↓ ossification		0.7 (6.3)	-	2.7 (16.7)	6.9* (47.1*)
		Manus	Score 3	64.8	73.8	49.0**	56.6
			Score 4	5.6	6.6	21.8**	18.6**
			Score 5	1.4	0.0	4.8	5.5
			Mean	2.80	2.86	2.99	3.15
		Pes	Score 1	93.7	96.7	86.4*	86.2*
			Score 2	6.3	3.3	13.6*	13.8*
			Mean	1.07	1.04	1.13	1.19
		No NOAEL identified in this study.					

Guideline developmental study in Alpk:APfSD rats

Mated female rats (24/sex/dose level) were gavaged with mesotrione at 0, 100, 300 or 1000 mg/kg bw/day on Days 7-16 of gestation. Animals were terminated on Day 22 of gestation.

No deaths occurred during the study period. Single incidences of piloerection, salivation, staining around the mouth and vaginal bleeding were noted in top dose animals. During the treatment period, bodyweight gain was lower in all treated groups (↓21% at the top dose), and mean bodyweights of 300 and 1000 mg/kg bw/d animals were slightly (↓<6%) but significantly lower than controls. Bodyweights in all groups increased post-dosing, however weights of top dose animals remained significantly lower than controls at termination (↓3.7%). Mean food consumption by all treated groups was significantly reduced during dosing. Food consumption increased post-dosing and values for Days 19-22 were comparable for all groups.

No macroscopic findings clearly attributable to treatment were noted in dams at necropsy. Mean fetal weights were decreased in all treated groups and attained statistical significance at 1000 mg/kg

bw/d (↓5.9%). No evidence of an effect on the number or survival of pups *in utero* was observed. Dose-related changes in the proportion of fetuses with minor skeletal defects and skeletal variants, indicative of reduced/delayed ossification were seen in all treated groups. Values for the majority of skeletal findings were outside the historical control range for the laboratory.

Non-guideline developmental study in Crl:CD-1(ICR)BR mice (range finding study)

Mated female mice (14/dose level) were gavaged with mesotrione at 0, 300 or 600 mg/kg bw/d on Days 7-16 of gestation. Animals were sacrificed on Day 19. Approximately half of the fetuses were examined for visceral and skeletal abnormalities, half were examined by serial sectioning and dissection.

Food consumption of top dose animals was significantly reduced from Day 1-3 (72% of controls), however food consumption during the remainder of the study was comparable in all groups. There were no significant effects on bodyweight.

No macroscopic findings attributable to treatment were noted at necropsy. The percentage of fetuses with minor skeletal abnormalities, indicative of reduced/delayed ossification, was increased at 600 mg/kg bw/d (without statistical significance).

Guideline developmental study in Alpk:Ap$_f$CD-1 mice

Mated female mice (30/dose level) were gavaged with 0, 10, 60, 150 or 600 mg/kg bw/d mesotrione on Days 5-18 of gestation. Mice were terminated on Day 19 of gestation.

Single incidences of diminished eye and ocular discharge were noted in one top dose animal. No further clinical signs of toxicity were noted. No effect was seen on the number, growth or survival of fetuses *in utero*.

Mean litter weights were slightly (but not significantly) higher in groups receiving mesotrione. Mean fetal weight was also increased in these groups, values attained statistical significance at 10 and 600 mg/kg bw/d (↑6.2% at both dose levels). The fetal and/or litter incidences of a number of minor skeletal defects, indicative of reduced or delayed ossification, were significantly increased at 600 mg/kg bw/d. Similarly, the incidences of long seventh rib (600 mg/kg bw/d) and ossified calcaneum (≥60 mg/kg bw/d) were significantly decreased. The toxicological significance of similar findings at lower dose levels is unclear in the absence of clear dose-response relationships and historical control data.

Guideline developmental study in New Zealand White rabbits

Mated female rabbits (20/dose level) were gavaged with mesotrione 0, 100, 250 or 500 mg/kg bw/d on Days 8-20 of gestation. Animals were terminated on Day 30.

One 100 mg/kg bw/d rabbit was found dead on Day 4 (prior to dosing). One 250 mg/kg bw/d animal was killed on Day 22 after exhibiting signs of toxicity and excessive weight loss. One 100 mg/kg bw/d animal (Day 29), two 250 mg/kg bw/d animals (Days 25 and 28) and two 500 mg/kg bw/d animals (Days 23 and 25) were killed following signs of abortion. Significant weight loss and clinical signs were seen in one 500 mg/kg bw/d animal prior to aborting. Mean bodyweights of 500 mg/kg bw/d animals were slightly lower than controls during the dosing period (↓2.3%), however were comparable to controls at termination. No significant effects were seen on food consumption.

No treatment-related macroscopic findings were noted in dams at necropsy. The numbers of implantations and live fetuses, litter and gravid uterus weights were slightly (but not significantly) reduced at 500 mg/kg bw/d. In the RAR, it states that these effects are thought to be a consequence

of the significantly higher pre-implantation loss seen in this group and are not therefore considered to be treatment-related. Mean fetal weights in all groups were comparable.

Significantly changed incidences of minor skeletal defects and skeletal variants indicative of altered ossification were seen in all treated groups, with the majority of findings within or slightly outside the historical range. The incidence of extra normal length 13th ribs and 27 pre-sacral vertebrae was in excess of the historical control range in all three dose groups. The number of litters with a mean *manus* score of 5 was significantly increased at 250 and 500 mg/kg bw/d. The numbers of litters with a mean *pes* score of 1 or 2 were significantly decreased at 500 mg/kg bw/d. The incidences of major skeletal defects, minor external and visceral defects were not affected by treatment with mesotrione.

Non-guideline developmental study in New Zealand White rabbits

The purpose of this study was to investigate the effects of mesotrione and tyrosine on developmental toxicity in the rabbit. Mated female rabbits (20/group) were gavaged with mesotrione at a dose level of 500 mg/kg bw/d on Days 8-20 of gestation. Animals were administered the test material in conjunction with diet containing 0 or 1% tyrosine. A further group gavaged with water was administered 1% tyrosine via the diet. Blood samples were taken pre-dosing and at 12 and 24 hours following dosing on days 8, 14 and 20 for the measurement of tyrosine concentration. Animals were terminated on Day 30. Urine samples were collected pre-test and on Day 18 and analysed for phenolic acids and tyrosine metabolites. Ophthalmoscopy was performed on Days 4-6 and 29. All animals were subject to gross necropsy; the liver and kidneys were assessed for TAT and HPPD activity. All fetuses were assessed for visceral and skeletal findings.

One animal receiving mesotrione and tyrosine (Group IV) was killed on Day 22 following decreased food consumption, weight loss and abortion. A number of clinical signs (skin sores, few/no faeces, and subdued behaviour) were noted with increased incidence in treated animals. Mean bodyweights of animals that received both mesotrione and tyrosine were slightly (but significantly) lower than controls from Day 14-20; weight loss was noted in a number of animals between Day 17 and 20. Recovery was seen on cessation of dosing. No treatment-related effects were seen on food consumption.

Plasma tyrosine concentrations were significantly increased in all treated groups as follows: mesotrione + tyrosine > mesotrione only > tyrosine only. Findings were most severe 12 hours post dosing, with evidence of recovery at 24 hours post dosing. Some evidence of adaptation was seen during the study, with a decrease in the 12-hour tyrosine levels seen in all groups. Values for plasma tyrosine levels at Day 29 showed virtually full recovery in all groups. Hepatic and renal HPPD activities were significantly decreased in groups administered mesotrione; renal TAT activity was also significantly decreased in these groups.

The number of major defects was increased in treated animals; single incidences of hydrocephalus, omphalocoele, spina bifida meningocoele and umbilical hernia were seen in animals dosed with both mesotrione and tyrosine. A number of blood vessel anomalies were also seen with increased incidence in treated groups. The incidence of a number of skeletal variants indicative of reduced/delayed ossification was increased in treated groups. *Manus* and *pes* ossification were similarly increased in treated groups.

4.11.2.2 Human information

No data are available.

4.11.3 Other relevant information

No data are available.

4.11.4 Summary and discussion of reproductive toxicity

The potential for mesotrione to induce effects on the integrity and performance of the male and female reproductive systems has been investigated in guideline and non-guideline studies in rats and mice. The potential for mesotrione to induce effects on the growth and development of the offspring, has been evaluated in rats (guideline study), mice and rabbits (guideline and non-guideline studies).

There were no effects on reproductive parameters.

In a guideline multigeneration study in rats, whole litter loss was increased in the F2 and F3CT(continued treatment) litters at the top dose (2500ppm). A decrease in the number of live born pups was also observed in these litters. Litter size was reduced in all groups and pup survival to day 22 was significantly decreased in F1, F2 and F3CT (continued treatment) generations at the top dose. No effect on pup survival was seen in the F3R (recovery) animals.

A non-guideline single generation study was conducted to investigate the role of tyrosine on reduced litter size, decreased pup survival and bilateral hydronephrosis in rats. Pregnant rats were fed diet containing 2500ppm mesotrione (96.8% purity) and/or varying concentrations of tyrosine (see Table 17 for details). In those rats receiving tyrosine only, plasma tyrosine levels increased with increasing dose (182.2, 199.5, 209.4 and 293.3 µM plasma tyrosine with 0, 0.5, 1 and 2% (w/w) dietary tyrosine). There were no treatment-related effects on pup survival, litter size or total litter loss in these groups. In animals dosed with 2500 ppm mesotrione and tyrosine, an increase in total litter loss and a decrease in pup survival was noted. In these animals, plasma tyrosine levels were much higher than in controls and in animals dosed with tyrosine alone. This suggests a correlation between the observed effects and the increased level of plasma tyrosine which results through the proposed mode of action (i.e., sustained inhibition of HPPD). However, it is noted that there was no clear relationship between plasma tyrosine levels and the increase in total litter loss and post/pre-natal deaths (i.e., plasma tyrosine levels were 2051, 2643 and 2010 µM with 2500ppm mesotrione and 0, 0.5 and 1% tyrosine with total litter loss of 0/18, 4/17 and 8/18 respectively).

No effects on the number or survival of pups *in utero* were observed in the rat developmental study, but it is noted that the treatment period in this study was much shorter.

Such effects were not seen in the two-generation study in the mouse or in the developmental studies with rabbits.

Effects in the kidney were noted in the multigeneration study and included bilateral hydronephrosis in pups of the F1, F2 and F3CT generations and renal pelvic dilatation in F1, F2R and F2CT adult females at ≥100 ppm. Effects in the eyes were also noted in the F1 and F2 pups at ≥ 100 ppm. These effects have been considered in the section on repeated dose toxicity.

In the developmental studies in the rat, mouse and rabbit there were a number of effects indicative of reduced/delayed ossification. Whilst the incidence of some findings exceeded the laboratory historical controls, the effects were variable and often occurred without a clear relationship to the dose. These effects are therefore not considered to be of sufficient concern for classification.

4.11.5 Comparison with criteria

A clear increase in total litter loss and the incidence of pre/post natal death was observed in the multi-generation and one-generation studies in the rat. The findings in the one-generation study are suggestive of a correlation between these effects and the level of plasma tyrosine in the dams; resulting from sustained dosing with mesotrione (i.e., through inhibition of HPPD).

However, whilst the available data are supportive of this conclusion, and whilst humans are expected to be less sensitive to the effects of mesotrione than rats (refer to section 2.2 in Part A), information is lacking regarding the relative potency for reproductive toxicity in humans. Therefore, there remains a degree of uncertainty regarding the relevance of these effects to humans. Given this uncertainty, it is proposed that classification with Repr 2; H361d – May cause damage to the unborn child is appropriate.

4.11.6 Conclusions on classification and labelling

Repr. 2; H361d: Suspected of damaging the unborn child

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

Neurotoxicity has been investigated in two guideline studies in rats; an acute study and a 90 day repeated dose study. These studies are summarised in Table 19. Further information about the studies (taken from the RAR) is provided below the table.

Table 19: Summary of relevant information on neurotoxicity

↑/↓ = increased/decreased compared to control. Unless otherwise stated, effects were seen in both sexes.

#The values for the NOAELs are provided for information only. All NOAEL values provided in this CLH Report have been taken from the Renewal Assessment Report (RAR), produced in accordance with Directive 91/414/EEC.

Method	Results and Remarks
Neurotoxicity study single dose OECD Guideline 424 GLP Rat (Alpk:APfSD Wistar) 10/sex/group Dose levels: 0, 20, 200 and 2000 mg/kg bw (nominal); oral (gavage) in deionised water Purity: 97.6% Exposure: Single dose Anonymous (1997u) RAR B6.7.1	No changes of toxicological significance at any dose level.

90 day neurotoxicity study OECD Guideline 424 GLP Rat (Alpk:APfSD Wistar), 12/sex/group Dose levels: 0, 2.5, 100 or 5000 ppm (nominal in diet) (equivalent to 0, 0.2, 8.3 and 403 mg/kg bw/d in males and 0, 0.23, 9.3 and 467 mg/kg bw/d in females) Purity: 97.6% Exposure: 90 days (Continuous in diet) Anonymous (1997v)	**2.5 ppm (0.20 / 0.23 mg/kg bw/day in males / females)** *Body weight*: Females↓ week 2 only (3.3%) **100 ppm (8.25 / 9.29 mg/kg bw/day in males / females)** *Clinical observations:* Eye opacities (3/12 males) *Body weight*: Females↓ throughout (2.7% at week 2 and week 14) *Food utilisation*: ↓ in females weeks 1 – 4 and 5 – 8 (3.1, 14.2%) *Ophthalmoscopy*: Corneal changes (hazy or complete opacity) with or without vascularisation at week 13 in 3/11 males and 1/12 females. *Gross pathology*: Opacity of the eye (1/5 males) **5000 ppm (402.8 / 466.64 mg/kg bw/day in males / females)** *Clinical observations:* Eye opacities (10/12 males, 4/12 females) *Body weight*: Males↓ 3.8%, females 6.8% at week 14 *Food consumption*: ↓ for males and females throughout (1.4 - 7.2%) *Food utilisation*: ↓ in males weeks 1 – 4 (6.4%) and females weeks 1 – 4 and 5 – 8 (14.1%, 23.7%) *Ophthalmoscopy*: Corneal changes (hazy or complete opacity) with or without vascularisation at week 13 in 10/12 males and 7/12 females. *Gross pathology*: Opacity of the eye (3/5 males and 1/5 females) #NOAEL for systemic toxicity = 2.5 ppm (0.2 or 0.23 mg/kg bw/day, for males and females respectively)

Guideline acute neurotoxicity study in Alpk:APfSD Wistar rats

Rats (10/sex/dose) received a single oral dose of 0, 20, 200 or 2000 mg/kg bw mesotrione. One male rat dosed at 200 mg/kg was found dead on day 5 but this was considered to be unrelated to treatment. There were no adverse clinical effects, no significant effects in the Functional Observational Battery and no effects on locomotor activity. There were no effects on brain weight, length or width and a comprehensive histopathological evaluation of the nervous system revealed no treatment related changes. Overall, no evidence of neurotoxicity was noted in this study.

Guideline subchronic neurotoxicity study in Alpk:APfSD Wistar rats

Rats (12/sex/dose) were fed diets containing 0, 2.5, 100 or 5000 ppm mesotrione for 90 consecutive days. One male rat in the 100 ppm group was killed for humane reasons in week 7 (not treatment related). Treatment-related effects on growth and associated reductions in food consumption and/or food utilisation were seen in both sexes at 5000 ppm and in females only at 100 ppm. Treatment-related corneal opacities were present at the end of the study in males at 100 ppm and in both sexes at 5000 ppm. There were no treatment related changes in any of the qualitative or quantitative components of the Functional Observational Battery. Locomotor activity for 100 and 5000 ppm males was slightly increased throughout the study; the changes were small and did not usually attain statistical significance, exhibit a coherent dose-response relationship or give any evidence of progression over the course of the study. In the absence of any clinical or histopathological evidence of neurotoxicity, these changes were considered to be of no

toxicological significance. There were no effects on brain weight, length or width and a comprehensive histopathological evaluation of the nervous system revealed on treatment related changes.

In the RAR, a NOAEL of 2.5 ppm (0.2 / 0.23 mg/kg bw/d) has been identified based on ocular findings at ≥100 ppm.

4.12.1.2 Immunotoxicity

Immunotoxicity has been investigated in one guideline study in mice. This study is summarised in Table 20. Further information about the study (taken from the RAR) is available below the table.

Table 20: Summary of immunotoxicity investigations

↑/↓ = increased/decreased compared to control. Unless otherwise stated, effects were seen in both sexes.

#The values for the NOAELs are provided for information only. All NOAEL values provided in this CLH Report have been taken from the Renewal Assessment Report (RAR), produced in accordance with Directive 91/414/EEC.

Method	Results and Remarks
Guideline immunotoxicity study (OPPTS 870.7800) Mouse (Crl:CD-1) 10 females/group Dose levels: 0, 500, 1500 or 5000 ppm mesotrione (equivalent to 0, 110.3, 332.4 and 1167.7 mg/kg bw/d) Purity: 83.0% Exposure: 28 days (Continuous in diet) All animals received single iv injection sheep red blood cells (sRBC) on day 24. Positive control: 50 mg/kg bw/day cyclophosphamide iv for 4 days OPPTS 870.7800 (1998) GLP Anonymous (2011) RAR: B.6.9.1	No treatment related effects at any dose level #NOAEL for general toxicity and immunotoxicity 5000 ppm (1167.7 mg/kg bw/day)

Groups of 10 female mice were fed diets containing 0, 500, 1500 or 5000 ppm mesotrione for 28 consecutive days. An additional group of 10 females served as positive controls, and received 50 mg/kg bw/d of cyclophosphamide by intravenous injection for the final 4 days. All animals received a single intravenous injection of the antigen, 1 x 10^8 sheep red blood cells (sRBC) on Day 24. There were no unscheduled deaths and no adverse signs of toxicity were recorded. At necropsy, there were no treatment related macroscopic findings but liver weights were higher in all groups receiving mesotrione and this reached significance in the mid and high dose groups (information on the magnitude of the increase is not provided in the RAR). Spleen and thymus weights were unaffected in all treatment groups. There was no statistically significant effect on spleen cell number or the humoral response. In the RAR, a NOAEL for general toxicity and sRBC mediated toxicity has been determined to be 5000 ppm (1167.7 mg/kg bw/day).

4.12.1.3 Specific investigations: other studies

No data are available.

4.12.1.4 Human information

No data are available.

4.12.2 Summary and discussion

In two guideline studies looking at neurotoxicity in rats (an acute study and a 90 day repeated dose study), no evidence for neurotoxicity was observed.

In a guideline immunotoxicity study in mice, no evidence for an immunotoxic effect of mesotrione was observed. Some effects on white blood cell parameters were noted in the repeated dose studies conducted in mice and dogs (see Section 4.7). However, the findings were not consistent across sexes, did not have a clear dose response and tended to be transient in nature. Therefore, these findings are not considered to represent an adverse effect of mesotrione on the immune system.

4.12.3 Comparison with criteria

No evidence for neurotoxic effect or an adverse effect on the immune system was observed in the available studies.

4.12.4 Conclusions on classification and labelling

Not classified, conclusive but insufficient for classification.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Note: In the following sections, technical mesotrione was often tested under its code name of 'ZA 1296' and is referred to as such.

5.1 Degradation

The following studies are available on the abiotic and biotic degradation and distribution of mesotrione. Unless otherwise stated, studies were conducted in accordance with the respective test guidelines, to GLP and were considered reliable.

Table 21: Summary of relevant information on degradation

Method	Results	Remarks	Reference
Aqueous hydrolysis			
EPA Pesticide Assessment Guidelines, Subdivision N, Series 161-1, 1989; GLP pH 5, 7, and 9; ca 1 mg a.s./L at 25°C Days of Application : 0, 5, 12, 19, 23 and 30 days	Stable to hydrolysis Transformation products: No major metabolites detected	Test material: [^{14}C-U-phenyl] and [^{14}C-2-cyclohexane] mesotrione Analytical purity: 97.8% and 98.2% Recoveries: 94.5-l 06.9% AR	Miles PD. & Powell S (1995) KCA 7.2.1.
OECD guideline 111 (guideline dated 1981) pH 4, 7, and 9; ca. 1 mg a.s./L at 50°C. Days of application:0, 2 and 5 days	Stable to hydrolysis Transformation products: No major metabolites detected	Test material: [^{14}C-U-phenyl]-mesotrione Analytical purity:98.9% Recoveries were 95.6-101.7% AR	
Aqueous photolysis			
EPA guidelines (Pesticide Assessment Guidelines, Subdivision N, Series 161-2, 1989)	Half-life (DT_{50}): 81-88 days under summer conditions at *ca.* 40 °N latitude Transformation products: No	Test material: [Carbonyl-^{13}C][phenyl-U-^{14}C] and [cyclohexane-2-^{14}C] mesotrione. Analytical purity 99.1% and 99.0%	Eya BK (1995) KCA 7.2.1.2
	Half-life (DT_{50}): 89-97 days under summer conditions at *ca.* 40° to 50°N latitude		Eya BK (1997) KCA 7.2.1.2
Indirect photochemical degradation			
OECD Guideline draft (Phototransformation of chemicals in water – Direct & indirect photolysis)	Half-life (DT_{50}): 20 days of summer sunlight (calculated for latitudes of 30, 40 and 50°N). Transformation products: A large number of degradates were formed. Levels of known metabolites observed were low with the main products being MNBA and AMBA.	Test material: [U-^{14}C]-Phenyl Mesotrione Purities: > 97% at initiation of irradiation for BPM-XXVIII and > 99% for CL-LVI-59	Oliver RG (2005) KCA 7.2.1.3/01

Method	Results	Remarks	Reference
Aerobic mineralisation in surface water			
OECD Guideline 309 (Aerobic Mineralisation in surface water – Simulation Biodegradation Test)	Non-sterilised: 60 DAT, resultant $DegT_{50}$ values 228 to 382 days. Sterilised samples, stable with 96% AR (mean) remaining at 60 DAT. Mesotrione was ultimately mineralised to carbon dioxide (< 1% AR). Transformation products: NOA437130 was the only metabolite found at ≥ 5% AR, reaching a maximum level of 9.7% (DAT 60)	Test Material: ^{14}C-phenyl ring labelled mesotrione Test concentration, (measured) (µg ai/L total water): 95 µg/L (38.78 kBq) or 10 µg/L (4.13 kBq) Analytical purity >97%	Graham R & Yeomans P (2013) KCA 7.2.2.2/01
Aerobic water/sediment			
EPA guidelines. (Pesticide Assessment Guidelines, Subdivision N, Series 162-4)	A DT_{50} for the dissipation of the metabolite AMBA from the water phase could not be calculated, however data available indicate rapid degradation for the first system and slower for the other.	Test material: [^{14}C-U-phenyl] and [^{14}C-2-cyclohexane] mesotrione Analytical purity >95%	Cary CA, Payne VA (1999) KCA 7.2.2.3 (original DAR AII, 7.2.1.2.2/01)
OECD 308	See Hardy (2013) for DT_{50} and DT_{50} values Transformation products: Yes Metabolites >5% AR (in aerobic and/or anaerobic conditions): AMBA, SYN546974 and SYN546935	[^{14}C-U-phenyl] and [^{14}C-2-cyclohexane] mesotrione Analytical purity >98%	Graham R & Gilbert J (2013a) KCA: 7.2.2.3/01
Kinetic evaluation of data from Cary CA, Payne VA (1999) and Graham R & Gilbert J (2013a)	Aerobic persistence endpoints: Geomean values: DT_{50} of 5.5 days (water) and 5.4 days (whole system) DT_{90} of 18.7 days (water) and 18.9 days (whole system) Anaerobic persistence endpoints: Geomean values: DT_{50} of 14.5 (whole system) DT_{90} 47.9 days		Hardy I (2013) KCA 7.2.2.3/01
Soil adsorption/desorption			
EPA PAG Subd. N 163-1 OECD guidelines	For all soils Kfoc ranged from 14 – 354 Worst case: Kfoc 14, 1/n 0.97 Arithmetic mean: Kfoc 83.3, 1/n 0.94 Median: Kfoc 47.5, 0.945	^{14}C-phenyl-labelled mesotrione ^{14}C-labelled mesotrione copper salt	Diaz DG (1995); Row D & Lane MCG (1997) KCA 7.1.3 Bramley YM, Pinheiro, SI & Verity AA (2002) KCA 7.1.3.1.1/02

5.1.1 Stability

Hydrolysis

Study 1: Miles PD, Powell S (1995); KCA 7.2.1.1

The hydrolytic stability of ZA 1296 was studied according to EPA guidelines (Pesticide Assessment Guidelines, Subdivision N, Series 161-1, 1989) and to US EPA GLP.

To study the rate of hydrolysis, [^{14}C-U-phenyl] and [^{14}C-2-cyclohexane] ZA 1296 were each added to separate samples of sterile aqueous buffer solutions (pH 5, 7, and 9; ca 1 mg a.s./L). Samples (25 mL) of each treated buffer were then incubated at 25°C in vials without traps for volatiles. At 0, 5, 12, 19, 23 and 30 days after application, single samples of each buffer were analysed for ZA 1296 by TLC. Radioactivity was quantified by LSC.

Recoveries were 94.5-106.9% AR for all treatments. For each pH, quantification of ZA 1296 gave similar results for each labelling site. ^{14}C-ZA 1296 was above the 95.2% AR for all treatments at all sampling times and no major metabolite was found.

The study authors therefore concluded that the compound is stable to hydrolysis in the conditions reported.

An additional 5 day, non-GLP study at 50°C according to OECD guideline 111 (guideline dated 1981) was also performed in order to further assess the degree of hydrolysis of ZA 1296.

[^{14}C-U-phenyl]-ZA 1296 was added to separate samples of sterile aqueous buffer solutions (pH 4, 7, and 9; ca. 1 mg a.s./L). Samples (25 ml) of each treated buffer were then incubated at 50°C in vials without traps for volatiles. At 0, 2 and 5 days after application, replicate samples of each buffer were analysed by TLC. Radioactivity was quantified by LSC.

Recoveries were 95.6-101.7% AR for all treatments. For each pH, quantification of ZA 1296 gave similar results. ^{14}C-ZA 1296 was above 91.7% AR for all treatments at all sampling times; just a slight increase in degradation was observed at pH 4 and 9 against pH 7 after 5 days. No major metabolite was found. The study authors have therefore concluded that the compound is stable to hydrolysis in the conditions reported.

Aqueous photolysis

Study 1: Eya BK (1995), Eya BK (1997) ; KCA 7.2.1.2

The aqueous photolysis of ZA 1296 was studied according to EPA guidelines (Pesticide Assessment Guidelines, Subdivision N, Series 161-2, 1989).

Two separate sterile aqueous buffer solution (pH 7) were prepared, containing [^{14}C-U-phenyl] ZA 1296 and [^{14}C-2-cyclohexane] ZA 1296 at concentrations of 2.24 and 2.15 mg a.s /L respectively. Aliquots (10 ml) were incubated at *ca.* 25°C in flasks fitted with traps for organic volatiles (ethyl acetate and ethanolamine) and CO_2 (NaOH). Replicate tubes were either kept in the dark or exposed to a xenon arc lamp with a special UV-filter to remove wavelengths below 290 nm for up to 19 (phenyl labelled samples) and 16 days (cyclohexane labelled samples). In a separate experiment, ZA 1296 was shown to have a significant adsorption above 290 nm. The average integrated light intensities applied to the two separate solutions were 528.6 and 529 watts/m^2 respectively. Duplicate samples for the [^{14}C-U-phenyl] ZA 1296 and [^{14}C-2- cyclohexane] ZA 1296 were taken respectively after 0, 1, 3, 7, 14, 16, 19 and 0, 1, 3, 7, 15, 16 days and correspondent dark controls after 3, 14, and 19 days and after 7 and 15 days respectively. Radioactivity was quantified directly by LSC and analysed by HPLC and TLC.

Total recovery was 94-102% and 97-101% AR for illuminated samples and correspondent dark controls. At the end of the studies, ZA 1296 accounted for a mean of 68-71% and 98-101% AR for the illuminated samples and dark controls respectively. No major metabolites were found in either sample. No degradates above 5% were found in the cyclohexane radiolabel photolysis, other than CO_2.

For the phenyl radiolabel photolysis, the largest degradate peak contained 6.25% of applied radioactivity. Degradates were not identified for the phenyl label since they were <10% and it is not possible from the data to determine whether any were >5% AR at two consecutive time points.

Carbon dioxide accounted for 20.2% AR after 16 days from the phenyl labelled samples, but was less than 10% AR from the cyclohexane samples.

The first order DT_{50} for photolytic degradation of ZA 1296 was calculated as 32-35 days, under test conditions. This was stated by the study author as equivalent to DT_{50} of 81-88 days under summer conditions at *ca.* 40 °N latitude.

To extrapolate the calculated DT_{50} to Northern Europe, the study author (Eya, 1997) has provided a conversion factor for the photolysis half-life from 40° to 50°N that leads to DT_{50} for ZA 1296 of 89-97 days for summer conditions in Northern Europe.

The conversion factor has been derived for methyl parathion validating the photolysis half-life calculated for summer at 50°N, on the basis of the extrapolation based on winter and autumn data, with the measured value provided in the reference paper (Mill *et al*, 1982).

Indirect photochemical degradation

Study 1: Oliver R & Edwards P (2005); KCA 7.2.1.3/01

In accordance with a draft OECD Guideline, ^{14}C-mesotrione (U-phenyl radiolabel) was applied to sterilised natural water (Middle Row Pond) at a concentration of ca. 8 µg/mL. The purities were > 97% at initiation of irradiation for BPM-XXVIII and > 99% for CL-LVI-59.

The treated samples were continuously irradiated using light from a xenon arc lamp (filtered to exclude <290 nm wavelengths), which was filtered to give a spectral distribution close to that of natural sunlight. The correlated colour temperature of the irradiation source is similar to daylight irradiation. The samples were maintained at a target temperature of 25 ± 2°C and irradiated for periods up to the equivalent of ca. 125 days Tokyo spring sunlight (which is equivalent to ca. 40 days of summer sunlight at latitude 50°N). Duplicate samples were analysed at 7 sampling intervals (only one replicate was analysed for the 15 days interval). The 7 DAT irradiated period was repeated on two separate occasions due to variability between the replicates and so to ensure a large enough sampling size to gain a more accurate mean. The repeat analysis of the 15 DAT interval was sampled 1 day early i.e. 14 DAT. Volatile radioactivity was continuously flushed from the photolysis vessels (air being pulled through using a peristaltic pump) and collected in liquid traps (two traps of 2M NaOH). Dark control samples were maintained at 25 ± 2°C and were analysed at an interval equivalent to the longest irradiated samples. Volatile radioactivity was continuously flushed from the dark control vessels (using a peristaltic pump) and collected in two 2M NaOH traps.

The pH of the natural water was determined to be 7.37, though there is insufficient information supplied to determine the pH level across the duration of the study.

Mesotrione underwent rapid and extensive photolysis in natural water. The degradation followed first order kinetics and the photolytic half-life was estimated to be ~12.8 days of continuous irradiation, which was equivalent to approximately 20 days of summer sunlight (calculated for latitudes of 30, 40 and 50°N).

The mean mass balance (across all irradiated samples) was 102.0% of the applied radioactivity. Mineralisation was also observed, with $^{14}CO_2$ representing 22.8% of the applied radioactivity at the end of the irradiation period.

A large number of degradates were formed. Levels of known metabolites observed were low with the main products being MNBA and AMBA. These reached maximum levels of 6.9% (7 DAT) and 3.9% (7 DAT) respectively. R282813 and R282470 were tentatively identified by co-chromatography. These reached maximum levels of 2.9% (7 DAT) and 3.5% (7 DAT) respectively. The majority of the remainder consisted of unretained polar materials (representing a maximum of 32.1% of the applied radioactivity at 25 DAT) when analysed using reversed phase HPLC with a polymer column. After isolation and sample concentration, the polar components were further resolved using the different retention mechanisms offered by a graphite column, with the maximum component reaching 10.5% of the applied radioactivity. This indicated that a large number of minor degradates were formed which is typical of the photolysis process. Attempts to identify some of these components, by co-chromatography with reference compounds and LC-MS-MS analysis, proved unsuccessful.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

Not expected to be readily biodegradable.

5.1.2.2 Screening tests

A study was not carried out in consideration of the fact that mesotrione (ZA 1296) is not expected to be readily biodegradable under the conditions recommended by the test guideline.

5.1.2.3 Simulation tests

Aerobic mineralisation in surface water

Study 1: Graham R & Yeomans P (2013); KCA 7.2.2.2/01

In accordance with OECD Guideline 309, the mineralisation rate and the route of degradation of ^{14}C-phenyl ring labelled mesotrione were investigated in Calwich Abbey natural water and natural water plus 0.02 g/L suspended sediment. Radio-labelled mesotrione (was applied to the water at nominal rates of 10 (4.13 kBq) and 95 µg/L (38.78 kBq) (low and high, respectively). The 95 µg/L rate was also applied to sterilised test systems (natural water and natural water plus 0.02 g/L suspended sediment). The systems were incubated under aerobic conditions and maintained in the dark at 20°C for up to 60 days. For each system, duplicate samples were taken for analysis at up to eight intervals.

At each sampling time, the quantity of radioactivity in the water was determined by liquid scintillation counting (LSC). A sub-sample of the water was then subjected to solid phase extraction (SPE) and eluted with acidified acetonitrile prior to LSC and chromatographic analysis. Any

volatile radioactivity was continuously flushed from the vessels, collected in traps and analysed. A mass balance was determined for each sample.

Separate reference samples (treated with sodium ^{14}C-benzoate at 10 µg/L) of natural water and natural water plus 0.02 g/L suspended sediment were prepared to determine whether a viable microbial population was present in the test system.

Separate blank control samples were similarly incubated to allow water quality measurements at each sampling interval.

The mean mass balances for the low and high test concentration natural water samples were 100 and 98% of applied radioactivity (AR) with ranges of 93 to 101 and 91 to 100%, respectively. The mean mass balances for the low and high test concentration natural water plus suspended sediment samples were 100 and 99% AR with ranges of 99 to 101 and 96 to 100%, respectively. The mass balances for the sterilised incubation groups were 100 and 99% AR for the water and water plus suspended sediment samples, respectively.

Over the duration of the study, the mean levels of parent compound decreased to between 80 and 84% AR for the water only and between 80 to 82% AR for the water plus suspended sediment. For the sterilised samples, the mean level of parent compound was 96% AR at 60 DAT. NOA437130 (MNBA) was the only metabolite found at ≥ 5% AR, reaching a maximum level of 10% AR at 60 DAT. NOA422848 (AMBA) and minor unknowns were also detected. Ultimately mesotrione was mineralised to carbon dioxide (< 1% AR).

The degradation rates ($DegT_{50}$) of mesotrione were determined using non-linear regression and a single first order kinetic model (SFO, KinGui, version 1.1).

The mineralisation rate and the route of degradation of ^{14}C-phenyl ring labelled mesotrione were investigated in Calwich Abbey natural water and natural water plus 0.02 g/L suspended sediment.

The mean mass balances for all incubation groups were 98 to 100% AR.

Throughout the duration of the study, little degradation was observed. The degradation was typically less than the desired level of 20% set out in OECD 309 (20% degradation from peak to lowest observed amount of active substance). Additionally, DAT 0 was not the time point with the highest observed amount for most of the study groups. The peak for Group A occurred at DAT 28 (98.4%, n =2), group B at DAT 21 (96.9%, n=2), and group E at DAT 21 (100.3%, n=1). The peak for Group F did occur at DAT 0 (97.0%, n=2).

Despite these limitations, acceptable kinetic fittings for the natural water and natural water and suspended sediment groups (A, B, E and F) were available.

For the non-sterilised, viable test systems, the mean levels of parent compound decreased to between 80 and 84% AR at the end of the incubation period (60 DAT), with resultant DegT50 values ranging from 228 to 382 days.

For the sterilised samples, mesotrione was found to be stable with 96% AR (mean) remaining at 60 DAT.

NOA437130 was the only metabolite found at ≥ 5% AR, reaching a maximum level of 9.7% (DAT 60, n=2). The maximum level for a single replicate was 11.3%, (DAT 60). The metabolite was present at >5% at two consecutive time points, and was increasing at the end of the study for all datasets. Consequently, no clear decline phases of the relevant metabolite NOA437130 were observed for any of the datasets sufficient for the purpose of deriving DT_{50} values.

NOA422848 and minor unknowns were also detected.

Ultimately mesotrione was mineralised to carbon dioxide (< 1% AR).

Water/sediment studies

Study 1: Cary CA & Payne VA (1999); KCA 7.2.2.3/01

Aerobic water/sediment studies were conducted according to EPA guidelines. (Pesticide Assessment Guidelines, Subdivision N, Series 162-4).

Samples of untreated water collected from the UK Virginia Water and Old Basing systems and respectively associated sand (pH 7.5, 0.5% OC) and sandy loam (pH 7.8, 7.5% OC) sediments were equilibrated in flasks for 29 days (200 ml water and 45 and 27 g sediment respectively; 2 mm sieved). Following equilibration, [^{14}C-U-phenyl] and [^{14}C-2-cyclohexane] ZA 1296 were each added in methanol to separate flasks (0.056 mg a.s./L). Treated flasks were purged with moistened air and fitted with traps for organic volatiles (carbon sieves) and for CO_2 (ethanolamine). All flasks were then incubated at 20°C in the dark for up to 101 days.

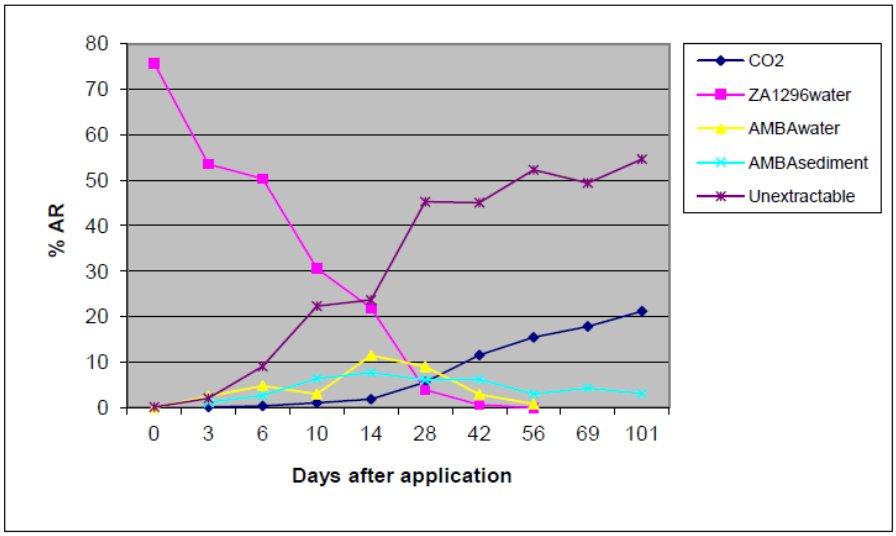
Duplicate flasks were removed at day 0 and nine representative time points: one was immediately analysed, the other was stored frozen to be eventually analysed further. Radioactivity in the water was quantified directly by LSC and analysed by TLC. Dissolved oxygen, redox potential and pH were also measured. Sediment was extracted twice with 0.05M ammonium hydroxide followed by acetonitrile; radioactivity in the extracts was quantified by LSC and analysed by one dimensional TLC; two dimensional TLC was adopted for two cyclohexane samples of the Virginia Water system to further investigate the non-discrete areas of radioactivity found.

Unextracted radioactivity was quantified by combustion and LSC. A further characterisation of the amount of radioactivity associated with the different sediment organic matter fractions was conducted. Radioactivity in the volatile traps was quantified by LSC.

Redox potential was 479-549 and 400-481 mV respectively for the Virginia Water and the Old Basing systems. Dissolved oxygen remained in the range 57-76 and 32-64% saturation respectively for the two systems. pH decreased from 7.2 to 6.8 and from 8.0 to 7.1 from day 0 to day 101 in the two systems respectively.

Total recovery from both systems was 88-109% AR and more than 82% of recovered ZA 1296 remained in the water phase at all sampling intervals. The maximum amount of ZA 1296 recovered from the sediment phase was 3.8% AR. Recovery of radioactivity from the Virginia Water system is given in Figure 2.

Figure 2: Recovery of radioactivity from the Virginia Water system



The pattern of recovery was similar for the Old Basing system, except for more rapid partitioning of radioactivity to sediment. At the end of the study, mean recovery of CO_2 and unextractable radioactivity was 17% AR and 69% AR respectively in the Old Basing system. In both systems, the cyclohexane labelled samples liberated more [^{14}C]CO_2 (27-28% AR) than the phenyl labelled ones (5-16% AR).

Unextractable radioactivity was shown mostly associated with the fulvic acid fraction (28% AR) in the Virginia Water system and the humin fraction (35% AR) in the Old Basing system.

The only major metabolite found was AMBA, which peaked at 19% AR (11.5% in the water phase) after 14 days in the Virginia Water system and at 17% AR (9.6% in the water phase) after 28 days in the Old Basing system. AMBA decreased to <1% AR after 56 days in the water phase of the Virginia Water system, but it was still accounting for 7.1% AR in the water of the Old Basing system after 101 days.

The metabolite MNBA was found only in the Virginia Water system, peaking in the water phase at 7.4% AR after 3 days and decreasing to <2% AR after l 0 days; it was just detected (<1% AR) once in the sediment phase. MNBA was only detected at >5% at one time point.

Maximum unidentified radioactivity recovered was 45 and 36% AR (cyclohexane labelled samples) in the Virgina Water and Old Basing system respectively. TLC analysis has shown this material split into a 'baseline' highly polar fraction and some non-discrete areas of radioactivity. In a further reverse phase TLC analysis of two cyclohexane labelled samples of the first system, no other major product was identified

The RMS under pesticides legislation (UK CRD) has recalculated the active substance DT_{50} according to FOCUS kinetic fitting methodology. Please refer to the evaluation of the new kinetic study Hardy (2013) (KCA 7.2.2.3/02) for further information.

A DT_{50} for the dissipation of the metabolite AMBA from the water phase could not be calculated, however data available indicate rapid degradation for the first system and slower for the other.

Study 2: Graham R & Gilbert J (2013a); KCA: 7.2.2.3/01

The rate and route of degradation of [Phenyl ^{14}C]-mesotrione was investigated according to OECD 308 in two different water-sediment systems: Calwich Abbey (silt loam) and Swiss Lake (sand). [^{14}C]-Mesotrione was applied to the water at a nominal rate equivalent to 150 g a.i./ha and an environmental water depth of 30 cm. For each water-sediment type, one set was maintained under aerobic conditions and one under anaerobic conditions. The systems were incubated in the laboratory and maintained in dark conditions at 20ºC for up to 102 days.

Duplicate samples from each system and incubation condition were taken for analysis immediately after treatment and at seven other intervals.

At each sampling time, the water phase was separated from the sediment phase and the sediment extracted with ammonium hydroxide followed by acetonitrile. Extractable ^{14}C-residues were characterized by HPLC and their quantification confirmed by TLC. Selected samples with high levels of unextracted residues were subjected to bound residue fractionation. Any volatile radioactivity was continuously flushed from the vessels, collected in traps and analysed. A mass balance was determined for each sample.

The surface water samples were analysed by LSC and concentrated for HPLC and TLC analysis.

Sediment samples were extracted with 0.05M ammonium hydroxide followed by acetonitrile. Sediment extracts were concentrated and analysed by LSC.

A representative unextracted sediment residue sample from each water-sediment type and each incubation condition was further extracted by acid reflux (pH 3, 4 hours) and organic matter fractionation. Previous method development showed that only a further 3% AR was removed by acid reflux and a further 12% AR removed (from a total 30% AR unextracted) during the basic extraction for organic matter fractionation for early time point samples. The resulting extracts were concentrated prior to HPLC analysis.

Structural assignment was initially made by co-chromatography with authenticated reference standards (where available). Confirmation of the presence of degradation products, where co-chromatography was demonstrated, was by LC-MS. Identification of WS-3 was supported by the mass spectroscopy data from a literature publication extraction, air-dried sediment samples were combusted in oxygen using a Harvey Biological Sample Oxidiser, model OX-500. The combusted products were absorbed in Carbo-Sorb® E: Permafluor® E+ 1:2 v/v and the radioactivity absorbed was determined by LSC.

A mass balance for each sample was determined by summation of the radioactivity recovered in the soil extracts, the total $^{14}CO_2$ evolved and the unextracted residues.

The percentage of applied radioactivity present as parent mesotrione in the water and in the total water-sediment system, determined using HPLC, was plotted against days of incubation and fitted to single first-order (SFO) kinetics using KinGUI version 1.1. The software calculated the dissipation and degradation rates and associated parameters.

Mean levels of parent compound in the water phase decreased from 99% AR at 0 DAT to < 1% AR at 102 DAT. NOA422848 was a major metabolite, reaching maximum levels of 16% AR and 6% AR at 46 DAT in Calwich Abbey and Swiss Lake water, respectively. SYN546974 was detected at up to 9% AR at 29 DAT in the Swiss Lake water but remained at < 5% AR in the Calwich Abbey water. All other metabolites, including SYN546935, R046791 and an unknown named WS-3 ($C_{14}H_{17}NO_8S$), individually accounted for < 5% AR.

Mean levels of parent compound in the sediment extracts increased to maximum values of 4 and 3% AR at 1 DAT, for the Calwich Abbey and Swiss Lake systems, respectively, before decreasing to 0% AR at 4 DAT. NOA422848 was a major metabolite, reaching a maximum level of 9% AR at 46 DAT in Calwich Abbey but remaining at < 5% AR in the Swiss Lake sediment. SYN546974 was detected at up to 6% AR at 14 and 46 DAT in the Calwich Abbey sediment and up to 26% AR at 102 DAT in the Swiss Lake sediment. All other metabolites, including SYN546935, R046791 and unknown WS-3, individually accounted for < 5% AR.

Mean levels of parent compound in the water phase decreased from 98% AR at 0 DAT to 0% AR at 102 DAT in the Calwich Abbey water and 0% AR at 7 DAT in the Swiss Lake water. NOA422848 was a major metabolite, reaching maximum levels of 14% AR at 102 DAT in Calwich Abbey and 8% AR at 4 DAT Swiss Lake water. SYN546935 was detected at up to 5% AR at 14 DAT in the Calwich Abbey water and up to 53% AR at 4 DAT in the Swiss Lake water. SYN546974 was detected at up to 5% AR at 46 DAT in the Calwich Abbey water and at up to 46% AR at 14 DAT in the Swiss Lake water. Unknown WS-3 was detected at up to 6% AR at 46 DAT in the Calwich Abbey water but remained at < 5% AR in the Swiss Lake water.

All other metabolites, including NOA437130, R046791, R282813 and R282470, individually accounted for < 5% AR.

Mean levels of parent compound in the sediment extracts increased to a maximum value of 3% AR at 7 DAT, for both systems, before decreasing to 0% AR at 14 DAT. NOA422848 was a major metabolite, reaching a maximum level of 8% AR at 102 DAT in Calwich Abbey but remaining at <5% AR in the Swiss Lake sediment. SYN546974 was detected at up to 5% AR at 46 and 102 DAT in the Calwich Abbey sediment and up to 27% AR at 102 DAT in the Swiss Lake sediment. All other metabolites, including SYN546935, R046791 and unknown WS-3, individually accounted for < 5% AR.

The RMS under pesticides legislation determined the dissipation rate (DT_{50}) from water and the degradation rate ($DegT_{50}$) of the parent in the total system using kinetic fittings, employing SFO, FOMC, and DFOP fits. The resulting DT50 values, in addition to kinetic parameter values and plots of visual fits and residuals, are available in the RMS evaluation of the notifier study KCA 7.2.2.3/02 (Hardy I, 2013 - see below).

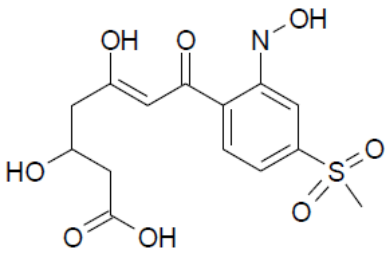
Conclusions

A number of discrete metabolites, individually accounting for < 5% AR in the total system, were detected, including R046791 (aerobic and anaerobic conditions), R282470 and R282813 (anaerobic conditions only).

The route of degradation was similar in both aerobic water-sediment systems with NOA422848 (AMBA) and SYN546974 being observed at > 5% AR. Several other degradation products were observed at low level, all less than 5% AR in either phase.

NOA422848 (AMBA), SYN546974 and SYN546935 were detected at >5% AR under anaerobic conditions. Unknown WS-3 (C14H17NO8S, structure below) was only detected at > 5% AR under anaerobic conditions for the Calwich Abbey water-sediment system. Several other degradation products were observed at low level, all less than 5% AR in either phase.

During the study, reasonable attempts were made to identify the unknown metabolite WS-3. This metabolite occurred above >5% applied radioactivity for two consecutive time points in the anaerobic Calwich system (5.4/7.8%).



The structure proposed by the applicant (above) cannot be conclusively confirmed.

Mineralisation (maximum 11% AR) and degradation to bound residues (maximum 61% AR) occurred under aerobic conditions.

Mineralisation (maximum 3% AR) occurred to a lesser extent under anaerobic conditions; however degradation to bound residues (59% AR) was comparable to that of aerobic conditions.

However, additional investigation on the unextracted residues showed that acid reflux extraction reduced the unextracted residues marginally and bound residue fractionation showed high levels of applied radioactivity associated with the insoluble humin fraction.

It should also be noted that the previous water/sediment study (Cary, 1999) used the radiolabel cyclohexane in addition to the phenyl radiolabel.

In the previous study, the cyclohexane data resulted in slightly longer DT_{50} values than the phenyl radiolabel (see kinetic analysis, below). However, it is the view of the RMS that the lack of cyclohexane label in the new water/sediment study does not represent a significant omission. Additionally, the statistical significance of the perceived difference between phenyl and cyclohexane degradation rate in the previous study has not been established.

For the active substance, both modelling endpoints using SFO kinetics and persistence endpoints using best-fit kinetic modelling have been calculated by the RMS using ordinary least squares optimisation. Please refer to the evaluation of the study Hardy (2013) (KCA 7.2.2.3/02), below, for further details.

Study 3: Hardy I (2013); KCA 7.2.2.3/02

DT_{50} values of both water/sediment studies (*Cary, 1999; Graham & Gilbert, 2013a*) have been calculated by the notifier in a kinetic analysis study. The RMS under pesticides legislation has recalculated these DT_{50} values according to FOCUS methodology.

Persistence endpoints were derived with total system and water compartment data, using FOCUS degradation guidance level P-1 (best fit model). This methodology yields total system DegT50 values and water column DT_{50} values (dissipation).

For modelling endpoints, the total system data was used for level P-1 using SFO kinetics. This yields system $DegT_{50}$ values suitable for use in computer modelling.

Level P-II (modelling of both water and sediment compartments simultaneously with transfer between each compartment) was not considered appropriate as the active substance was not present in the sediment at significant levels (maximum individual replicate, 4.3% - Calwich aerobic system). Mesotrione was not found in the sediment beyond sampling time day 7.

Where available, replicates were utilised in the kinetic fitting (multiple values at each time point, not a single averaged value). The RMS did not utilise radiolabels as replicates; data from each radiolabel was modelled separately.

For water DT50 determination, any radioactivity present in the sediment at time 0 was added to the amount present in the water column. Any radioactivity determined as metabolites at time 0 was also added to the water column.

Table 22: Aerobic persistence endpoints

Location	Radiolabel	Compartment	Kinetic fit	DT_{50} (days)	DT_{90} (days)	X2
Basing	Phenyl	Water	FOMC	2.4	8.9	5.3
		Total system	FOMC	2.4	9.2	5.6
	Cyclohexane	Water	SFO	4.2	13.8	13.3
		Total system	SFO	4.2	13.8	13.3
Virginia	Phenyl	Water	SFO	5.3	17.5	13.5
		Total system	FOMC	4.8	17.4	6.4
	Cyclohexane	Water	SFO	7.0	23.2	13.4
		Total system	SFO	7.2	24.1	14.1
Calwich	Phenyl	Water	SFO	6.7	22.2	3.4
		Total system	SFO	6.6	21.8	4.5
Swiss	Phenyl	Water	DFOP	10.6	39	2.4
		Total system	DFOP	10.5	38.8	2.7
GEOMEAN				**5.5 (water dissipation)** **5.4 (whole system degradation)**	**18.7 (water dissipation)** **18.9 (whole system degradation)**	

Under anaerobic conditions, dissipation rates/degradation rates (DT_{50}/$DegT_{50}$) for mesotrione of 14 days and 1 day were observed in the Calwich Abbey and Swiss Lake water-sediment systems, respectively. DT_{50} values calculated from the anaerobic Swiss Lake data were not appropriate however, as the kinetic fitting featured poor visual fits and unacceptable chi squared values. The Swiss lake data points are indicative of a similarly short DT_{50} under anaerobic conditions.

Table 23: Anaerobic persistence endpoints

Location	Radiolabel	Compartment	Kinetic fit	DT_{50} (days)	DT_{90} (days)	X2
Calwich	Phenyl	Water	SFO	14.5	48.2	9.21
		Total system	SFO	14.4	47.7	8.75
Swiss	Phenyl	Water	SFO*	1.36	4.52	24.2
		Total system	SFO*	1.47	4.86	23.8
GEOMEAN				**14.5**	**47.9**	

* Unacceptable fit. Chi squared value too high, or fitting failed (no standard error or C.I figures available). All kinetic fits (including biphasic kinetics), fail.

5.1.3 Summary and discussion of degradation

Hydrolytic degradation: Mesotrione was stable to hydrolysis at 25°C and 50°C at all environmentally relevant pHs. Hence, mesotrione is not subject to significant aqueous hydrolysis.

Direct photochemical degradation: Direct photolysis is not the major surface water degradation pathway.

Indirect photochemical degradation: Mesotrione is considered to undergo rapid photolysis in natural water, however the relevance of this to surface waters throughout Europe and all times of the year is unclear in relation to CLP degradation criteria.

Ready biodegradability: A ready biodegradability study has not been required or conducted. Mesotrione is classified as not biodegradable by default.

Aerobic mineralisation in surface water: Little degradation was reported, being typically less than the desired level of 20% set out in OECD 309. The metabolite present at >5% AR was MNBA.

Water/sediment studies: The aerobic persistence endpoints were as follows: Geomean DT_{50} values of 5.5 days (water) and 5.4 days (whole system) with DT90 values of 18.7 days (water) and 18.9 days (whole system). The anaerobic persistence endpoints are as follows: Geomean DT_{50} of 14.5 and DT_{90} of 47.9 days. The main metabolite was AMBA.

Overall, from a hazard classification perspective and in relation to CLP criteria, mesotrione is considered to be 'not rapidly degradable'.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

Study 1: Diaz DG (1995); KCA 7.1.3 (DAR II, 7.1.2/01)

A batch equilibrium adsorption/desorption study was conducted for ZA 1296 in compliance with EPA guidelines (Pesticide Assessment Guidelines Subdivision N, Series 163-1, 1982) and OECD guidelines (Guidelines for the testing of chemicals, 1993).

[^{14}C-U-phenyl] ZA 1296 in 0.01 M calcium chloride (10 mL) was added to non-sterile samples (4 g) of five soils at concentrations of 0.02, 0.2, 1.0, 5.0 mg a.s./L for each soil (except Wisconsin

soil 0.14 mg/L not 0.2 mg/L). A soil:solution ration of 1:2.5 was maintained. Treated slurries were shaken in 'Teflon' tubes at 20 ± 2 °C for 24 hours in the dark (equilibrium confirmed in pre-test). After equilibration, the supernatant was removed by centrifugation and radioactivity quantified by LSC. The resultant soil pellets were re-suspended in 0.01 M calcium chloride (equal to decanted volume) and again equilibrated, then analysed as above.

To test for ZA 1296 stability, the 5.0 mg/L treatment solution and the highest treated adsorption and desorption supernatant solutions were analysed by HPLC, along with soil extracts (the soil was extracted with NaOH, a portion of which was acidified with HCl to pH 0-1 then partitioned with ethylacetate). ZA 1296 was detected at >96% of the radioactivity in the adsorption supernatant, 93% of the radioactivity in the desorption supernatant and 90% of the radioactivity in the soil extract (exception 81% in Garonne soil extract; if other 19% is one component this is < 0.9% AR).

Total radioactivity recovered ranged from 89-102% AR. The adsorption and desorption isotherms for each concentration were used to calculate Freundlich coefficients (Kf) and Kf_{oc} values for each soil, which are given in Table 24. All values were calculated without correction for the slight decomposition of ZA 1296 detailed above.

The 1/n values were not reported in the original DAR or study report, but are needed for use as endpoints in the FOCUS models. For the purpose of renewal under EU pesticides legislation the RMS has calculated these for adsorption using the original C*e* solution and *x/m* soil values, given below for each soil. These are in agreement with those calculated by the applicant for renewal (except for a slight difference in 1/n value for Visalia sandy loam which the applicant reported as 0.94 and the RMS calculated as 0.959).

Table 24: Freundlich exponent values calculated by the RMS for adsorption

Soil type	% oc	pH	Adsorption		1/n
			Kf	Kfoc	
Wisborough Green silty clay loam	2.63	5.1	4.46	170	0.902
Wisconsin silt loam	1.58	6.2	0.74	47	0.921
Toulouse clay	1.79	6.5	1.25	70	0.915
Garonne loam	1.03	7.8	0.15	14	0.971
Visalia sandy loam	0.53	8.2	0.13	25	0.959

Study 2: Rowe D & Lane MCG (1997); KCA 7.1.3 (DAR II, 7.1.2/02 ; III, 9.2.1.1/03)

A batch equilibrium adsorption/desorption study was conducted for ZA 1296 in compliance with EPA guidelines (Pesticide Assessment Guidelines Subdivision N, Series 163-1, 1982) and OECD guidelines (Guidelines for the testing of chemicals, 1993).

[^{14}C-U-phenyl]ZA 1296 in 0.01 M calcium chloride (20 mL) was added to duplicate sterile samples (10 g) of four soils at concentrations of 0.05, 0.1, 0.2, 1.0 and 2.0 mg a.s./L for each soil. Treated slurries (soil: water ratio 1:2 i.e. 10 g soil: 20 ml 0.01M $CaCl_2$) were shaken in 'Teflon' tubes at 20 ± 2°C for 16 hours in the dark (equilibrium confirmed in pre-test). Control samples were prepared with no soil to test for adsorption to the tubes. After equilibration, the supernatant was removed by centrifugation and radioactivity quantified by LSC, therein the supernatant was frozen. After centrifugation, soil pellets were re-suspended in 0.01 M calcium chloride (equal to decanted

volume) and equilibrated again (for 16 hours), then analysed as above. Residual radioactivity in selected soil samples was quantified by combustion/LSC.

ZA 1296 stability was checked *via* TLC of the aqueous phase extract and the soil extracts (soil extracted with acidified methanol) for all soils treated at 0.2 mg /L and additionally at all test concentrations for the Garronne soil. ZA 1296 was found at > 90% of the radioactivity in the adsorption and desorption aqueous phase extracts (exception 83% in Eastern Region Technical Centre (ERTC) and 86 % in Garonne (1.0 and 2.0 mg/L only) desorption extracts). In the soil extracts, ZA 1296 was typically 80-90% of the radioactivity found, though it did fall to 68% for Wisconsin adsorption soil extract.

Total radioactivity recovered ranged from 92-104% AR. The adsorption and desorption isotherms for each concentration were used to calculate Freundlich coefficients (Kf) and Kfoc values for each soil, which are given in Table 25 for adsorption. After a single desorption step, desorption Kf were 0.28 to 1.7 and desorption Kf_{oc} values were 33-130. All values were calculated without correction for the slight decomposition of ZA 1296 detailed above.

Mass balance and % extract determined as mesotrione by TLC analysis were both reported for each concentration in one soil ('Garonne) and at a single concentration (0.2 µg/mL) for the other 3 soils. Mass balance was acceptable at >91.5%AR. The % extract as unchanged parent in aqueous phase was also acceptable at >89.4%AR. Some degradation of parent had occurred in the soil extracts (with 68% and 74.4% extract as parent in the Wisconsin and Garonne soils). However, concentrations were measured directly in both the soil and aqueous phases and used to calculate Kf and Kf_{oc} values and it was assumed for these calculations that all applied radioactivity was parent.

The RMS under pesticides legislation notes that the average % adsorbed was less than the recommended 20% for two of the soils (15 and 8.1% average adsorbed for Garonne and ERTC soils, respectively), but the soil to solution ratio tested was already 1:2 and a higher soil to solution ratio (such as 1:1) may not have been feasible. The RMS considers that this is not unexpected for a weakly sorbed compound and as such is not a critical issue.

The 1/n values were not reported in the original DAR or study report, but are needed for use as endpoints in the FOCUS models. For the purpose of renewal under EU pesticides legislation the RMS has calculated these for adsorption using the original C*e* solution and *x/m* soil values, given below for each soil. These are in agreement with those calculated by the applicant for the purpose of renewal.

Table 25: Equilibrium solution and soil concentrations (average of duplicate values) after 24 hours (adsorption)

Soil type	% oc	pH	Adsorption		1/n
			Kf	Kf_{oc}	
Wisconsin silt loam	1.28	6.1	0.61	48	0.947
ERTC sandy loam	0.58	6.4	0.33	58	0.950
Pickett Piece clay loam	3.31	7.1	0.97	29	0.932
Garonne loam	0.87	7.7	0.16	19	0.954

Study 3: Bramley YM, Pinheiro SI and Verity AA (2002); KCA 7.1.3.1.1/02

The adsorption properties of ^{14}C-phenyl-labelled mesotrione (radiochemical purity 97.4%) and ^{14}C-labelled mesotrione copper salt were studied in four soils of varying organic matter contents and pH values: Champaign (silty clay loam), Garonne (sandy clay loam), Wisconsin (silt loam) and Pickett Piece (clay loam), using a standard batch equilibrium method, in the dark at 20°C.

This study was conducted according to OECD Guideline 106 ('Adsorption - Desorption using a Batch Equilibrium Method', January 2000) and was carried out in two stages:

Stage One: soil adsorption coefficients Kd and K_{oc}, together with the Freundlich adsorption constants Kf and Kf_{oc}, were determined for Champaign soil. Champaign soil was treated at 5 nominal concentrations (0.05, 0.1, 0.2, 1.0 and 2.0 µg/mL).

Stage Two: only Kd and K_{oc} values were determined. Garonne, Wisconsin and Pickett Piece soils were all treated at one nominal concentration (0.1 µg/mL).

The experiment was conducted at two soil:aqueous ratios, 1:1 and 1:2. The soil and $CaCl_2$ solution was sterilised by gamma irradiation. Based on existing data for mesotrione, an adsorption equilibration time of 24 hours was used. Champaign silty clay loam soil (USDA classification) was air-dried and 2mm sieved before being stored in plastic bags at 4°C until required. Soil samples were pre-equilibrated for 24 hours in 'Teflon' tubes with 0.01M $CaCl_2$ solution. Each tube contained 10 g soil (dry weight) and aqueous $CaCl_2$ was added to a volume of 10 mL for 1:1 ratio, or to 20 mL for 1:2 ratio.

Treatment rates were at concentrations of 0.05, 0.1, 0.2, 1.0 and 2.0 µg/mL. Treated slurries were shaken (*ca* 22-25 rpm) for 24 hours at 20 ± 2°C. Supernatants were separated by centrifugation and removed by pipette for analysis. Remaining supernatant and soil pellets were stored frozen until analysis. Aqueous solutions were quantified by liquid scintillation counting (LSC) and analysed by thin layer chromatography (TLC). Soil pellet was further extracted with 0.05M ammonium hydroxide followed by acetonitrile; these soil extracts were quantified by LSC and also analysed by TLC.

Actual measured application rates were 0.05, 0.10, 0.19, 0.97 and 1.94 µg/mL.

Control tubes with no soil were included to test for adsorption of mesotrione to test vessels. Blank samples (soil and $CaCl_2$ solution only) were also prepared as background samples for LSC quantification.

Average recoveries of radioactivity at the end of the experimental phase for Champaign soil treated with mesotrione at 1:1 ratio were 101.4-103.9%, and for the 1:2 ratio were 93.3-101.8%. TLC analysis was conducted on representative aqueous supernatants and on selected soil extracts to demonstrate that mesotrione was essentially stable under the experimental conditions. The percentage radioactivity identified as parent mesotrione in the aqueous phase extract was 79.4% (1:1 ratio) and 83% (1:2 ratio). In the soil extracts the range of percentage radioactivity determined as parent mesotrione was 84-90.2% for both ratios. For the purpose of calculation of adsorption coefficients, applied radioactivity in soil and aqueous phase was assumed as parent. At 1:1 and 1:2 ratios for mesotrione >87.2% and >76.4% of applied radioactivity was adsorbed.

The RMS under pesticides legislation was not able to find the measured concentrations in the aqueous phase after the adsorption step in the study report. The values given in the table were actual rates of application instead. The RMS initially back-calculated the concentrations in the aqueous phase from the Kd values reported (Kd /concentration adsorbed to soil) to check the Kf,

Kf_{oc} and 1/n values given. The RMS results are reported below, with the main difference that the RMS derives a 1/n of 0.93 and not 0.79 for the mesotrione 1:1 ratio. The applicant subsequently confirmed the missing aqueous concentrations, which match those back-calculated by the RMS. However, the RMS still derives a different Kf, Kf_{oc} and 1/n for the 1:1 ratio.

There was only very weak correlation of Kf value with soil %om or %oc (r^2 0.2) and none with clay content (r^2 0.095) based on the values below.

Conclusion and summary of Kf_{oc} values for mesotrione

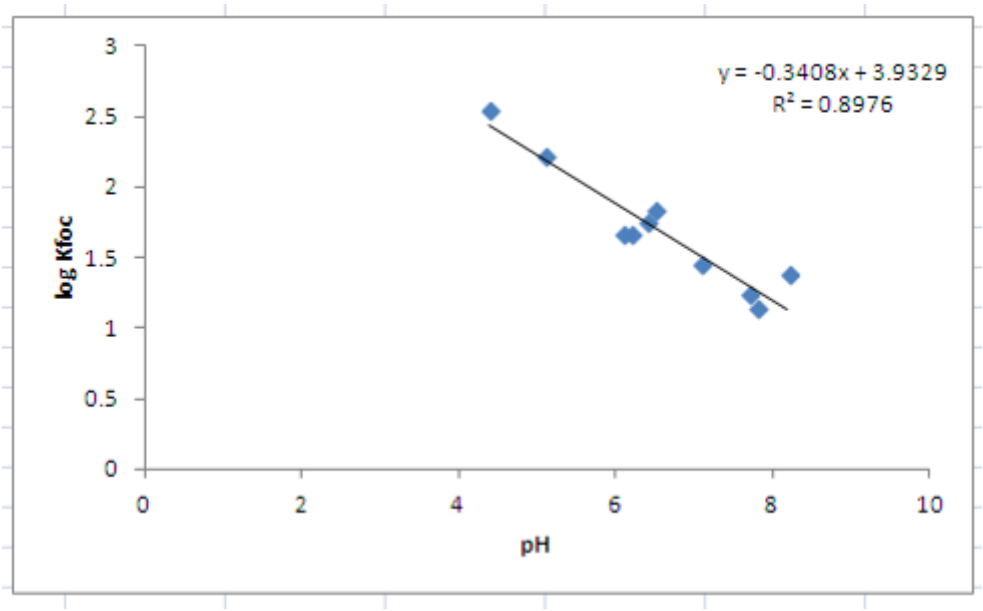
Table 26: Freundlich adsorption coefficients Kf_{oc} for mesotrione

Soil Location	Soil Type	Soil pH (H_2O)	% Organic Matter	clay	KF (L/kg)	KF_{OC} (L/kg)	1/n	Reference
Wisconsin	Silt loam	**6.2**	2.72	25.2	0.74	47	**0.92**	Diaz (1995) KCA 7.1.3 (DAR II, 7.1.2/01)
Visalia	Sandy loam	**8.2**	0.92	12.4	0.13	25	0.94 **(0.96)**	
Wisborough	Silty clay loam	**5.1**	4.53	31.2	4.48 (4.46)	171 (170)	**0.90**	
Toulouse	Clay	**6.5**	3.08	52.4	1.25	70	**0.92**	
Garonne	Loam	**7.8**	1.78	24.4	0.15	14	**0.97**	
ERTC	Sandy loam	6.5 **(6.4)**	1.0	7.6	0.33	57 (58)	**0.95**	Rowe & Lane (1997) KCA 7.1.3 (DAR II, 7.1.2/02; III, 9.2.1.1/03)
Garonne	Loam	**7.7**	1.5	21.6	0.16	18	**0.95**	
Wisconsin	Silt loam	**6.1**	2.2	24.8	0.61	48	**0.95**	
Pickett Piece	Clay loam	**7.1**	5.7	33.2	0.97	29	**0.93**	
Champaign (1:2 ratio)*	Silty clay loam	**4.4**	3.0	34	6.16	354	**0.94**	Bramley (2002) KCA 7.1.3.1.1/02
						pH dependent		
Worst case						**14**	**0.97**	
Arithmetic mean						**83.3**	**0.94**	
Median						**47.5**	**0.945**	

Values in parentheses where RMS determined a slightly different value to applicant. Values in italics for Gunma volcanic ash excluded as not representative of EU agricultural soils. Values in bold used in mean.

The RMS has accepted 1:2 soil:solution results here for Champaign soil. If 1:1 soil: solution ratio results (Kf_{oc} 365 l/kg and 1/n of 0.93) are added for the extremely low pH Champaign soil, then two high Kf_{oc} values for this same soil weight the arithmetic mean Kf_{oc} towards a less conservative 108.9 l/kg, though the mean and median 1/n value and median Kf_{oc} are similar. The applicant also did not include this 1:1 ratio value possibly because they arrived at a different 1/n of 0.79. (Without the data from Bramley 2002, the mean Kf_{oc} would be 53 l/kg with 1/n 0.939, median Kf_{oc} 47 l/kg with 1/n 0.947).

Figure 3. Relationship of log(K_{FOC}) vs pH for mesotrione



The Kf_{oc} values above were converted to logKf_{oc} values and plotted against soil pH. The relationship between Kf_{oc} and pH for mesotrione is defined by the equation shown where x represents pH. The 90th and 10th percentile soil pH values for maize cropping area in Europe from the GIS study (Fish, 2013) were pH 5.1 and 7.9. From this Kf_{oc} values have been derived at pH 5.1 and 7.9 to represent acidic and alkaline sorption values for modelling with the median 1/n of 0.94.

At pH 5.1 y = -0.3408 x 5.1 + 3.9329 = Kf_{oc} of 156.61 L/kg

At pH 7.9 y = -0.3408 x 7.9 + 3.9329 = Kf_{oc} of 17.40 L/kg

The median 1/n is 0.94.

The worst case Kf_{oc} is 14 L/kg with corresponding 1/n of 0.97.

These K_{oc} values would indicate mesotrione to be highly mobile in most soil systems and to predominantly be found (and undergo primary degradation) in the water phase, as indicated in the water-sediment studies evaluated above.

5.2.2 Volatilisation

Mesotrione has low volatility (<5.7 × 10^{-6} Pa at 20°C) and is shown to have insignificant volatilisation from soil and plants.

The photochemical oxidative degradation of mesotrione in air is rapid

Half-life 1.5 days calculated using Atkinson method, 12 hour day, 1.5 × 10^{6} OH/cm^{3}. Therefore long-range transport is considered not to be of relevance.

The predicted environmental concentration in air is therefore predicted to be negligible.

5.2.3 Distribution modelling

No relevant information.

5.3 Aquatic Bioaccumulation

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

A number of log P_{ow} values are quoted in Table 8 for mesotrione from studies by Goodman, M. In unbuffered water these range from 0.11 to 0.32 and all buffer values (pH 5-9) are negative values (≤ -1.0). These Log P_{ow} values are all below the CLP cut-off value of 4.

5.3.1.2 Measured bioaccumulation data

No studies on potential bioconcentration of active substances with a log Pow < 3 (under EC Reg. 1107/2009) are required.

5.3.2 Summary and discussion of aquatic bioaccumulation

For mesotrione the log P_{ow} values are ≤ 0.32 and therefore below the CLP cut-off value of 4. This indicates a low potential for bioaccumulation.

5.4 Aquatic toxicity

The following studies are available on the toxicity of mesotrione to aquatic life. Unless otherwise stated, studies were conducted in accordance with the respective test guidelines, to GLP and were considered reliable.

Table 27: Summary of relevant information on aquatic toxicity

Method	Results	Remarks	Reference (+re-analyses)
Fish			
Rainbow trout (*Oncorhynchus mykiss*) Acute (static) EPA Pesticide Assessment Guideline Subdivision E, Section 72-1 (≈ OECD 203) Nominal concentrations: 0 and 120 mg a.s./L	96 hour LC_{50} >120 mg/L [S, Nom]	Mesotrione Purity 95.1% w/w	Anonymous (1994d) KCA 8.2.1/01
Bluegill sunfish (*Lepomis macrochirus*) Acute (static) EPA Pesticide Assessment Guideline Subdivision E, Section 72-1 (≈ OECD 203) Nominal concentrations: 0 and 120 mg a.s./L	96 hour LC_{50} >120 mg/L [S, Nom]	Mesotrione Purity 95.1% w/w	Anonymous (1994)e KCA 8.2.1/02
Fathead minnow (*Pimephales promelas*) Chronic (flow-through) USEPA Guideline 72-4; GLP Nominal concentrations of 0, 12.5, 25, 50, 100 and 200 mg a.s./L	36 day NOEC: 12.5 mg/L [Nom] (physical symptoms*) 36 day EC_{10} : ND 36 day EC_{20} : ND	Mesotrione Purity 97.6% w/w	Anonymous (1997), Anonymous (2013) KCA 8.2.2.1/01
Aquatic invertebrates			
Water flea *(Daphnia magna)* Acute (static) OECD Guideline 202 Part I Nominal concentrations: 0, 130, 216, 360, 600 and 1000 mg a.s./L	48 hour EC_{50} >622 mg/L [M, X]	Mesotrione Purity 96.8% w/w	Gentle & Hamer, 1995 KCA 8.2.4.1/01

Water flea *(Daphnia magna)* Chronic (semi-static) ASTM E1193-87 Standards Guide; OECD Guideline 202; GLP Nominal test concentrations 0, 100, 180, 320, 560 and 1000 mg a.s./L	21 day NOEC 180 mg/L [M] (reproduction & length) EC_{10} : ND EC_{20} : ND	Mesotrione purity 96.8% w/w	Morris *et al.*, 1996 (Liedtke, 2013a) KCA 8.2.5.1/01
Algae			
Green microalgae *(Pseudo-kirchneriella subcapitata)* Chronic (static) EPA Pesticide Assessment Guideline Subdivision J, Section 123-2 (≈ OECD 201) Nominal concentrations: 0, 0.38, 0.75, 1.5, 3.0, 6.0, 12, 24 and 49 mg a.s./L	72-hr E_bC_{50}: 4.5 mg/L 72-hr E_rC_{50}: 13 mg/L 72-hr $NOEC_{b,\,r}$: 0.75 mg/L 72-hr E_bC_{10}: 0.692 mg/L 72-hr E_bC_{20}: 0.958 mg/L all Nom	Mesotrione Purity 95.1% w/w	Shillabeer, Kent & Smyth, 1997 (Dark, 2013) KCA 8.2.6.1/01
Navicula pelliculosa Chronic (static) EPA Pesticide Assessment Guideline Subdivision J, Section 123-2 (≈ OECD 201) Nominal concentrations: 0, 0.75, 1.5, 3.0, 6.0, 12, 24, 48 and 96 mg a.s./L	72-hr E_bC_{50}: 68 mg/L 72-hr E_rC_{50}: 66 mg/L 72-hr $NOEC_{b,\,r}$: 48 mg/L 72-hr E_rC_{10}: 51.0 mg/L 72-hr E_rC_{20}: 53.2 mg/L all Nom	Mesotrione Purity 97.6% w/w	Smyth *et al.*, 1997a (Dark, 2012) KCA 8.2.6.2/02
Aquatic macrophytes			
Gibbous duckweed (*Lemna gibba*) 14 days, chronic (semi-static) EPA Pesticide Assessment Guideline Subdivision J, Section 123-2 Nominal concentrations: 0, 0.50, 1.0, 2.0, 4.0, 8.0, 16, 32 and 64 µg a.s./L	EC_{50} (for frond no.): 0.022 mg/L EC_{50} (for dry weight): 0.0077 mg/L E_rC_{50} (growth rate, frond no.): 0.0599 mg/L **E_rC_{50} (growth rate, dry weight): 0.0257 mg/L** NOEC (for frond no.): 0.008 mg/L **NOEC (for dry weight): 0.002 mg/L** E_rC_{10} (growth rate, frond no.): 0.0068 mg/L **E_rC_{10} (growth rate, dry weight): 0.002 mg/L** E_rC_{20} (growth rate, frond no.): 0.015 mg/L E_rC_{20} (growth rate, dry weight): 0.0047 mg/L all Nom	Mesotrione Purity 97.6% w/w	Smyth *et al.*, 1997b (Anonymous, 2013b) KCA 8.2.7/01

Gibbous duckweed (*Lemna gibba*) 7 days, chronic (semi-static) OECD Guideline 221and US EPA/OPPTS 850.4400. Nominal concentrations: 0, 2.0, 4.0, 8.0, 16, 32 and 64 µg a.s./L	**E_rC_{50} (growth rate, frond no.): 0.028 mg/L** **E_rC_{50} (growth rate, dry weight): 0.028 mg/L** **NOE_rC (growth rate, frond no.): 0.002 mg/L** **NOE_rC (growth rate, dry weight): 0.002 mg/L** all Nom	Mesotrione Purity 86.1% w/w	Kosak L. & Wydra V., 2015

Endpoints in **bold** are considered below for use in acute and chronic hazard classification Nom – based on nominal test concentrations
M – based on mean measured concentrations
ND – could not be determined
S – single test concentration and a dilution control were used
X – All concentrations used in the test on *Daphnia magna*, apart from the lowest, were above the water solubility. For determination of EC50 see below
* – Physical symptoms were loss of balance, less activity, spinal deformity, skin lesions and internal bleeding
CORR1 – In the original DAR this study was erroneously referred to as Kent *et al*., 1997

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Study 1 : Anonymous (1994d) ; KCA 8.2.1/01

Rainbow trout (*Oncorhynchus mykiss*) were exposed to a single concentration of ZA 1296 (purity 95.1% w/w) in freshwater in a static test system for 96 hours at nominally 12°C. The study was conducted to EPA Pesticide Assessment Guideline Subdivision E, Section 72-1 (which is equivalent in key respects to OECD 203). The nominal ZA 1296 concentration was 120 mg/l. A control group of fish were exposed to dilution water only. Actual concentrations of ZA 1296 were determined by chemical analysis at 0, 48 and 96 hours. Records of mortality and symptoms of toxicity were made at 0, 24, 48, 72 and 96 hours.

There were no mortalities or symptoms of toxicity in the fish exposed to 120 mg ZA 1296 per litre. Analysis of the test solutions confirmed that the mean measured concentration was 120 mg/L of ZA 1296.

The nominal 96 hour LC_{50} value for ZA 1296 to rainbow trout was >120 mg/L and the no observed effect concentration (based on symptoms of toxicity) was ≥120 mg/L.

Study 2 : Anonymous (1994e); KCA 8.2.1/02

Bluegill sunfish (*Lepomis macrochirus*) were exposed to a single concentration of ZA 1296 (purity 95.1% w/w) in freshwater in a static test system for 96 hours at nominally 22°C. The study was conducted to EPA Pesticide Assessment Guideline Subdivision E, Section 72-1 (which is equivalent in key respects to OECD 203). The nominal ZA 1296 concentration was 120 mg/L. A control group of fish were exposed to dilution water only. Actual concentrations of ZA 1296 were determined by chemical analysis at 0, 48 and 96 hours. Records of mortality and symptoms of toxicity were made at 0, 24, 48, 72 and 96 hours.

There were no mortalities or symptoms of toxicity in the fish exposed to 120 mg ZA 1296 per litre. Analysis of the test solutions confirmed that the mean measured concentration was in good agreement (108%) with the nominal concentration.

The nominal 96 hour LC_{50} value for ZA 1296 to bluegill sunfish was >120 mg/L and the no observed effect concentration (based on symptoms of toxicity) was ≥120 mg/L.

5.4.1.2 Long-term toxicity to fish

Study 1 : Anonymous (1997w) ; Cambridge Environmental Assessments (2013); KCA 8.2.2.1/01

In a 36-day fish early life stage flow-through test conducted in accordance with USEPA Guideline 72-4 and GLP, fathead minnow (*Pimephales promelas*) eggs were exposed at 25°C to nominal concentrations of ZA 1296 (purity 97.6%) of 12.5, 25, 50, 100 and 200 mg a.s./L and to a dilution water control. The effects on hatchability, survival, growth and on the appearance of physico-chemical symptoms in fish in early life stage were evaluated.

Four replicates of 20 eggs (less than 24 hours old, generated within the laboratory) were used for each concentration and for the dilution water control. Nominal test solution flow rate and nominal flow loading were respectively 40 mL/min and 120 mL/egg/hour.

Exposure levels were monitored for each test concentration and for the dilution water control on exposure days 0, 2, 3, 18, 24 and 31. Daily observations of larval mortality, behaviour, and appearance were made and any abnormal effects recorded. The test was terminated after 32 days post-hatch (hatch identified at exposure day 4).

Larvae were fed three times a day during the week and twice a day at weekends. Feed was freshwater rotifers (3 ml culture/tank), rotifers and brine shrimps (1.33 mL/fry/feed) since post-hatch day 4, brine shrimp only (at increasing rates from 2 to 3 mL/fry/feed) since post-hatch day 8; a high protein pelleted fish food replaced one brine shrimp feed since post-hatch day 16. No food was provided in the last two days of the study.

The test was conducted in tap water of hardness 39-55 mg/L as $CaCO_3$; dissolved oxygen was 6.8-8.2 mg/L, temperature 24.2-25.1°C and pH 6.6-7.9.

Mean measured concentrations ranged from 88 to 98% of the nominal values. Results were based on nominal concentrations.

Percentage hatchability and survival were respectively 80-100% and 84-100%; no significant differences were found between replicates and controls; NOEC for hatchability and survival was 200 mg a.s./L. Growth (length and weight) was a more sensitive indicator of toxicity. Significant decreases in length and weight were observed at 50 and 200 mg a.s./L and a significant decrease in length at 100 mg a.s./L against the corresponding control. No significant decrease in overall growth (length and weight) was found at 25 mg a.s./L. NOEC for growth was 25 mg a.s./L. The most sensitive indicator of toxicity, however, was the appearance of physical symptoms. On post-hatch day 10 single fry showed loss of balance at 100 mg a.s./L. More severe symptoms were observed on post-hatch day 25, with fry less active than the control, spinal deformities and lesions around the tail for a number of individuals at 200 mg a.s./L, spinal deformity and lesion around the head for a single fry at 100 mg a.s./L and internal bleeding for a single fry at 25 mg a.s./L. From day 28 to the end of the study loss of balance, spinal deformities and skin lesions in the tail area increased for replicates at 50, 100, 200 mg a.s./L. On the basis of symptoms seen at concentrations of 25 mg/L and higher, the NOEC for physical symptoms was 12.5 mg a.s./L.

In the statistical re-analysis of the study (Anonymous, 2013), the EC_{10} and EC_{20} for hatchability, post-hatch survival and weight could not be calculated. The EC_{10} for length was considered unreliable. Consequently, no reliable EC_{10} or EC_{20} estimates could be calculated from the measured parameters.

For CLP purposes this is not required since an NOEC of 12.5 mg/L is already established.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Study 1: Gentle & Hamer (1995); KCA 8.2.4.1/01

In accordance with OECD Guideline 202 Part I, *Daphnia magna* (first instar, <24 hours old) were exposed to ZA 1296 (purity 96.8% w/w) in a static system for 48 hours at 20°C. The test concentrations were 130, 216, 360, 600 and 1000 mg ZA 1296 per litre. The control group of animals were exposed to dilution water only.

Actual concentrations of ZA 1296 to which the *Daphnia* were exposed were determined by chemical analysis at 0 and 48 hours. *Daphnia* were assessed for effects in terms of signs of toxicity and immobility at 24 and 48 hours.

The measured concentrations were in close agreement with the nominal concentrations (104-107%) although all concentrations used, apart from the lowest, were above the water solubility. Assessment of the highest concentration was only possible at 48 hours due to the preparation being cloudy. Therefore, only a 48 hour EC_{50} value was determined, which was 900 mg/l (95% confidence interval 622 to 1042 mg/L), based on mean measured concentrations. The 48 hour mean measured no observed effect concentration (NOEC) was 622 mg/L.

Although the 48 hour EC_{50} value of ZA 1296 to *Daphnia magna* was originally reported as 900 mg/L, cloudiness due to precipitation was seen at the highest concentration, nominally 1000 mg/L where the measured concentration was 996 mg/L at 48 h. At the next highest concentration, 600 mg/L and below, analysis indicated that concentrations were maintained. Mortality was 93% at 1000 mg/L and 0% at 600 mg/L. The study author stated the EC50 to be 900 mg a.s./L, however, because of solubility problems the evaluator considers it is more appropriate to base the EC50 on the next lowest concentration. The EC_{50} is, therefore, > 622 mg a.s./L based on mean measured concentration. In the list of endpoints for the earlier pesticide Review Report on mesotrione (14 April 2003, SANCO/1416/2001 - Final), the value of >900 mg a.s./L was misreported for acute invertebrate toxicity.

5.4.2.2 Long-term toxicity to aquatic invertebrates

Study 1: Morris DS *et al*. (1996); Liedtke A (2013a); KCA 8.2.5.1/01

The chronic toxicity of technical ZA 1296 (purity 96.8% w/w) to *Daphnia magna* was assessed in a 21-day semi-static study performed according to ASTM E1193-87 and OECD Guideline 202.

Daphnia were exposed to nominal test concentrations of ZA 1296 of 100, 180, 320, 560 and 1000 mg a.s./L and to a dilution water control, to evaluate the effects on survival, reproduction and growth. For each exposure level and for the corresponding control, ten laboratory bred first instar Daphnia housed in separate vessels were adopted. The cultures were fed algae and microencapsulated food. Test vessels were covered glass beakers containing 80 ml medium. The

test medium was prepared by dissolving the test material directly in dilution water (of hardness 237 mg/L as $CaCO_3$). The test was conducted under a 16 hours light: 8 hours dark photoperiod at 20 ± 1°C; mean dissolved oxygen was 8.5 and 8.4-8.9 mg/L, respectively, for the control and the test solutions; mean pH (freshly prepared solutions) ranged from 3.8 (1000 mg a.s./L solution) to 7 (100 mg a.s./L solution), mean pH of the dilution water control was 8.1.

Test solutions were renewed three times a week. Concentrations of ZA 1296 were measured for each new test solution and for the replaced one.

Observations for the presence of offspring were made daily for each vessel from exposure day 5. The numbers of live and dead juveniles present in the reproduction vessels were counted at each renewal. Length and dry weight of adults surviving in the replicates were measured at the end of the exposure period.

Mean measured concentrations ranged from 97 to 100% of nominal for test solutions at 100 and 180 mg/L. For test solutions at 320, 560 and 1000 mg/L, concentrations measured at the beginning of the study were respectively 94, 60 and 37% of nominal; no further measurements of concentrations for these test solutions were provided. Results were expressed as measured concentrations.

There were no mortalities of parent *Daphnia* in the dilution water control and the 180 mg A.S. /L test concentration. 100% mortality after 1 day was observed for the 300, 340, 370 mg A.S. /L measured concentrations. The LC_{50}, based on measured concentration, was 230 mg a.s. /L (95% C.L. 190-340 mg/L).

In the dilution water control and at concentrations up to 180 mg a.s./L reproduction was not significantly reduced and 4 broods were completed. NOEC for reproduction based on mean measured concentration was 180 mg a.s./L. There was no significant difference in the mean length of *Daphnia* in either the 97 or 180 mg a.s./L concentration compared with the control; mean length observed was 4 mm. NOEC for growth (length) based on mean measured concentration was 180 mg a.s./L. Significant reduction of 46% and 18% in dry weight were observed respectively at 97 and 180 mg a.s./L mean measured concentrations. The study authors did not propose a NOEC for dry weight, due to the variability in dry weight observed in this study and to the overall reliability of this endpoint. Therefore, the NOEC based on reproduction and length is 180 mg a.s./L.

This GLP study was conducted in accordance with ASTM and OECD guidelines. However, the study was not entirely in line with modern standards as several concentrations tested exceeded the water solubility. The pH was also lower than recommended in the higher concentrations. However, despite these shortcomings, the study is considered sufficiently reliable as effects and the NOEC occurred at concentrations where no problems with solubility and pH were noted.

In the statistical re-analysis of the study (Liedtke, 2013a), the EC_{10} and EC_{20} for reproduction rate, length and body weight of the daphnids after 21 days could not be determined due to the low toxic effect of the test item and the absence of a concentration-effect relationship at the two lowest test concentrations of 100 mg/L and 180 mg/L. At the highest mean measured concentrations of 300, 340 and 370 mg/L, 100% mortality occurred at observation day 1 so sub-lethal effects were not relevant. However, a reliable NOEC is established which is sufficient for CLP classification purposes.

5.4.3 Algae and aquatic plants

Study 1: Shillabeer, Kent & Smyth (1997); Dark R (2013); KCA 8.2.6.1 / 01

The effects of ZA 1296 on the green alga *Selenastrum capricornutum* (now known as *Pseudokirchneriella subcapitata*) were investigated according to EPA Pesticide Assessment Guideline Subdivision J, Section 123-2 (which is equivalent in key respects to OECD 201). *S. capricornutum* was inoculated at 0.320×10^4 cells/mL and cultured in concentrations of ZA 1296 technical (purity 95.1% w/w) in sterile culture medium at nominally 24°C for 120 hours under static conditions. The nominal concentrations employed were 0 (control), 0.38, 0.75, 1.5, 3.0, 6.0, 12, 24 and 48 mg ZA 1296 per litre.

Actual concentrations of ZA 1296 were determined by chemical analysis at 0 and 120 hours. Algal cell numbers were determined with a Coulter counter at 24, 48, 72, 96 and 120 hours.

The measured concentrations were in close agreement (within ±20%) with the nominal concentrations and therefore the results are given on the basis of nominal concentrations of ZA 1296. The 72 hour EC_{50} values are given in accordance with the OECD 201 guideline requirement and CLP guidance.

The nominal 72 hour EbC_{50} and ErC_{50} values for ZA 1296 to *Selenastrum capricornutum* (*P. subcapitata*) were 4.5 and 13 mg a.s./L respectively and the 72 hour NOEC (for both biomass and growth rate) was 0.75 mg a.s./L.

In the statistical re-analysis of the study on mesotrione to *Pseudokirchneriella subcapitata* (Dark, 2013), the mean average specific growth rate in the control over the initial 72 hour test period was determined to be 1.66/day (a 144 fold increase). The coefficient of variation of average specific growth rate in the control cultures over the whole test period was 0.659 %. The mean coefficients of variation for section-by-section specific growth rates in the control cultures were 10.5 % (0 to 72 hours), 11.1 % (0 to 96 hours) and 24.8 % (0 to 120 hours).

The calculated 72-hr EC_{10} and EC_{20} values were 0.926 and 0.1.66 mg mesotrione/L, respectively, based on average specific growth rate. For classification purposes it is proposed to still refer to the nominal 72-hr NOErC of 0.75 mg a.s./L.

Study 2: Smyth DV *et al*. (1997a); Dark R (2012) ; KCA 8.2.6.2 / 01

The effects of ZA 1296 on the freshwater diatom, *Navicula pelliculosa*, were investigated according to EPA Pesticide Assessment Guideline Subdivision J, Section 123-2 (which is equivalent in key respects to OECD 201). *N. pelliculosa*, was inoculated at 0.300×10^4 cells/mL and cultured in concentrations of ZA 1296 (purity 97.6% w/w) in sterile culture medium at nominally 24°C for 120 hours under static conditions. The nominal concentrations employed were 0 (control), 0.75, 1.5, 3.0, 6.0, 12, 24, 48 and 96 mg ZA 1296 per litre.

Actual concentrations of ZA 1296 were determined by chemical analysis at 0 and 120 hours. Algal cell numbers were determined with a Coulter counter at 24, 48, 72, 96 and 120 hours.

The measured concentrations were in close agreement (within ±20%) with the nominal concentrations and therefore the results are given on the basis of nominal concentrations. The 72 hour EC_{50} values are given in accordance with the OECD 201 guideline requirement and CLP guidance to allow for comparison with the EC_{50} values for ZA 1296 on *Pseudokirchneriella subcapitata* (*S. capricornutum*).

The nominal 72 hour EbC_{50} and ErC_{50} values for ZA 1296 to *Navicula pelliculosa* were 68 and 66 mg/L respectively and the 72 hour no observed effect concentration (for both biomass and growth rate) was 48 mg/L.

In the statistical re-analysis of the study on mesotrione to *Navicula pelliculosa* (Dark, 2012), the mean average specific growth rate in the control over the 72 hour test period was reported to be 1.23/day (a 40 fold increase). The coefficient of variation of average specific growth rate in the control cultures over the test period was 1.62 %. The mean coefficient of variation for section-by-section specific growth rates in the control cultures were 102 % (0 to 72 hrs), 78.5% (0 to 96 hours) and 76.6 % (0 to 120 hours).

The lowest calculated 72-hr EC_{10} and EC_{20} values were 51.0 and 53.2 mg a.s./L, respectively, for average specific growth rate. For classification purposes it is proposed to still refer to the nominal 72-hr NOErC of 0.48 mg a.s./L.

Study 3: Smyth DV *et al*. (1997b); Anonymous (2013b); KCA 8.2.7 / 01 p111

The effects of ZA 1296 on duckweed, *Lemna gibba*, were investigated according to EPA Pesticide Assessment Guideline Subdivision J, Section 123-2. *L. gibba*, was cultured in concentrations of ZA 1296 (purity 97.6% w/w) in sterile culture medium at nominally 25°C for 14 days under static conditions. Replicate test vessels initially contained 3 plants, each with 4 fronds. Test solutions were renewed on days 5 and 9. The nominal concentrations employed were 0 (control), 0.50, 1.0, 2.0, 4.0, 8.0, 16, 32 and 64 µg ZA 1296 per litre.

Actual concentrations of ZA 1296 were determined by chemical analysis on days 0, 5, 9 and 14. Assessments were made at 2, 5, 7, 9, 12 and 14 days by counting the number of fronds per test vessel. The dry weight of the total number of fronds per test vessel was determined at the end of the study.

Although some individual samples at the lowest concentrations were below the limit of detection, the mean measured concentrations ranged from 93-103% of nominals and were in close agreement (within ±20%) with the nominal concentrations, therefore the results are given on the basis of nominal concentrations.

The 14-day nominal EC_{50} values for frond growth (frond number) and dry weight of ZA 1296 to *Lemna gibba* were 0.022 and 0.0077 mg a.s./L respectively. The 14-day no observed effect concentration for frond growth was 0.008 mg a.s./L and for dry weight was 0.002 mg a.s./L. Endpoints at 7 days were not determined, however growth in controls appeared to continue satisfactorily up to 14 days.

In the statistical re-analysis of the study on mesotrione to *Lemna gibba* (Anonymous, 2013b and subsequently by the Applicant, pers. comm. 2016), the following 14-day EC_{10}, EC_{20} and EC_{50} endpoints for growth rate and yield based on frond number and dry weight were determined:

Table 28: EC_{10}, EC_{20} and EC_{50} estimates based on nominal concentrations for *Lemna gibba* exposed to mesotrione

EC values [µg/L]	Parameter based on:			
	Frond numbers		Dry weight of the plants	
	Growth rate	Yield	Growth rate	Yield
14-day EC_{10}	6.8	5.6	2.0	1.4
95% CL	3.8-9.7	3.0-7.7	1.2-2.8	0.86-1.8
14-day EC_{20}	15	7.9	4.7	2.2
95% CL	10-19	5.1-10	3.4-6.1	1.6-2.8
14-day EC_{50}	59.9	-	25.7	-
95% CL	29.4-468.63*		15.3-56.9*	

* The Applicant claims that the goodness of fit for the E_rC_{50} recalculations was unsatisfactory - see comment below

For classification purposes, the most relevant of these recalculated endpoints for acute classification is the nominal 14-day E_rC_{50} value for growth rate of 0.0257 mg a.s./L based on dry weight. Although very similar, this is preferred to the original value from Smyth *et al*. of 0.022 mg/L based on frond growth (number) alone rather than on growth rate. Also, for chronic classification the E_rC_{10} value for growth rate of 0.002 mg a.s./L based on dry weight, is the same as the lowest nominal 14-day NOEC for dry weight of 0.002 mg/L from the original study. Either value may therefore be used for chronic classification.

When undertaking the E_rC_{50} recalculations for growth rate, the Applicant suggested that the goodness of fit for the effects in this study was not satisfactory. This is borne out by the wide confidence limits seen for the E_rC_{50} based on frond number in particular. Effects on frond no. also did not follow a consistent concentration-dependant trend. Whilst the MSCA does not necessarily consider this study by Smyth *et al*. to be wholly unreliable, it may not be especially accurate. The Applicant has therefore, submitted a new 7-day *Lemna* study from 2015 which was not included in the original mesotrione RAR (see Study 4 below).

Study 4: Kosak L. & Wydra V. (2015). ibacon GmbH Report No. 105732240, Syngenta file No. ZA1296_10438)

The effects of ZA 1296 (purity 86.1% w/w) on duckweed, *Lemna gibba*, were investigated according to OECD Guideline 221 (2006) and US EPA/OPPTS 850.4400 (1996). This was a 7-day semi-static growth inhibition test with a subsequent 3-5 week recovery period in clean water/growth medium (which is not discussed further here). During the exposure phase, the medium was renewed on days 3 and 5. Replicate test vessels initially contained 3 plants, each with 4 fronds (i.e. 12 fronds, ave. dry weight 1.5 mg per vessel). Environmental conditions were: temperature: 23-24°C; pH in fresh media: 7.5-7.9, in aged media: 8.5- 9.0; continuous illumination: 7300-7770 Lux. Test solutions were renewed on days 5 and 9. The nominal concentrations employed were 0 (control), 2.0, 4.0, 8.0, 16, 32 and 64 µg ZA 1296 per litre.

Actual concentrations of ZA 1296 were determined by chemical analysis on days 0 and 7. Assessments of frond numbers per test vessel were made on days 0, 3, 5 and 7; the dry weight of fronds per test vessel was determined at the end of the study.

At the start of the test, the concentrations of the test item were found to be in the range 93-107% of the nominal values and at the end of the test they were 87-122%. Although slightly outside of 80-

120% (122%) in just one concentration at 7-days, nominal concentrations were used for the calculation and reporting of results, this is considered acceptable by the eMSCA.

For frond number, the 7-day EC_{50} for yield (E_yC_{50}) and growth rate (E_rC_{50}) for ZA 1296 to *Lemna gibba* were 6.0 and 28 µg/L respectively, based on nominal concentrations. For dry weight, the 7-day EC_{50} for yield (E_yC_{50}) and growth rate (E_rC_{50}) were 5.2 and 28 µg/L respectively, based on nominal concentrations. The 7-day NOEC (for both growth rate and yield) was determined to be 2.0 µg/L. Only the growth rate values are used for hazard classification, i.e. the 7-day E_rC_{50} for both frond number and dry weight of 28 µg/L (0.028 mg/L) and the 7-day NOE_rC of 2.0 µg/L (0.002 mg/L).

Results for mean frond number and dry weights are presented in the following table along with the respective growth rate inhibition and estimated EC_{50} and NOEC values:

Table 29: Effect of mesotrione (ZA 1296) on growth rate (frond number and dry weight) of *Lemna gibba*

Nominal concentration (µg/L)	**Mean No. fronds/replicate (day 7)**	**Dry Weight (mg) (day 7)**	**Based on Frond Number (0-7 days)**		**Based on Dry Weight (0-7 days)**	
			Growth Rate	**Inhibition of Growth Rate (%)**	**Growth Rate**	**Inhibition of Growth Rate (%)**
Control	250.0	32.8	0.434	-	0.440	-
2	246.3	32.5	0.431	0.6	0.439	0.3
4	138.7	16.9	0.349*	19.6	0.345*	21.7
8	107.0	11.2	0.312*	27.9	0.287*	34.8
16	66.0	8.0	0.243*	43.9	0.238*	45.9
32	49.0	7.5	0.200*	53.8	0.230*	47.8
64	43.7	5.6	0.184*	57.7	0.188*	57.3
E_rC_{50} µg/L			28		28	
95% confidence limits			20 - 37		19 - 42	
NOE_rC			2.0		2.0	

* mean value significantly different from the control (tested with Williams Test, $\alpha = 0.05$, one-sided)

5.4.4 Other aquatic organisms (including sediment)

None submitted or required.

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

Based on the environmental fate and behaviour data available for technical mesotrione, the substance is stable to hydrolysis and it does not readily biodegrade or ultimately degrade sufficiently rapidly to either CO_2 or to unclassified degradants in whole water-sediment systems. According to CLP criteria, the substance is therefore considered to be 'not rapidly degradable' for hazard classification purposes. The substance also has a Log Pow of ≤ 0.32 and is considered to be not bioaccumulative for CLP classification purposes.

Sufficient reliable acute and chronic/long-term ecotoxicology data are available on each of the main aquatic trophic groups (fish, invertebrates and algae) along with higher aquatic plants/macrophytes (*Lemna*), which are relevant given the herbicidal activity of mesotrione. Based on acute L/EC50 endpoints >>1 mg/L as well as chronic NOECs >0.1 mg/L, neither fish nor invertebrates (*Daphnia*) are sensitive to mesotrione in comparison with the endpoints for algae and aquatic plants. The classification proposals will therefore focus on the toxicity to these more sensitive groups.

Acute aquatic hazard classification:

The most acutely sensitive alga/diatom species tested is *Pseudokirchneriella subcapitata*, with a 72-hr E_rC_{50} of 13 mg mesotrione/L. However, the aquatic macrophyte *Lemna gibba* is notably more sensitive with a 14-day E_rC_{50} for dry weight of 0.0257 mg/L in the study by Smyth *et al*., 1997b (recalculated by Anonymous, 2013b and Syngenta, 2016) or a very similar 7-day E_rC_{50} for frond number or dry weight of 0.028 mg/L in the study by Kosak L. & Wydra V., 2015.

These *Lemna* EC_{50} values of 0.0257 to 0.028 mg/L are <0.1 mg/L and are in the range >0.01 to <0.1 mg/L, therefore mesotrione should be classified according to CLP hazard criteria as: 'Aquatic Acute Category 1' (H400) with an Acute M-factor of 10.

Chronic aquatic hazard classification:

The most chronically sensitive alga/diatom species tested is *Pseudokirchneriella subcapitata*, with a 72-hr NOE_rC of 0.75 mg mesotrione/L. However, the macrophyte *Lemna gibba* is more sensitive with a 14-day NOE_rC for dry weight of 0.002 mg/L in the study by Smyth *et al*., 1997b or the identical 7-day NOE_rC values for frond number or dry weight of 0.002 mg/L in the study by Kosak L. & Wydra V., 2015. These NOE_rCs are supported by the lowest E_rC_{10} value also of 0.002 mg/L recalculated by Anonymous, 2013b from the study by Smyth *et al*., 1997b.

These *Lemna* NOE_rC or E_rC_{10} values of 0.002 mg/L are between >0.001 and <0.01 mg/L and, since mesotrione is considered 'not rapidly biodegradable' according to CLP criteria, it should be classified as: 'Aquatic Chronic Category 1' (H410) with a Chronic M-factor of 10.

5.6 Conclusions on classification and labelling for environmental hazards

Aquatic Acute 1; H400: Very toxic to aquatic life;

Acute M-factor = 10

Aquatic Chronic 1; H410: Very toxic to aquatic life with long lasting effects;

Chronic M-factor = 10

5.7 Hazardous to the ozone layer

5.7.1 Short summary and overall relevance of the provided information on ozone layer hazard

This end point is not addressed in this CLH proposal.

6 OTHER INFORMATION

No other relevant information.

7 REFERENCES

A number of references within this CLH report are considered to be confidential. These references are indicated by "Anonymous (XXXXx)" in the text, and can be found in full in the confidential Annex.

Renewal Assessment Report:

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Renewal Assessment Report (RAR – Mesotrione – Volume 3, Annex B.6; Toxicology and Metabolism – November 2015)

Renewal Assessment Report (RAR – Mesotrione – Volume 3, Annex B.8; Environmental Fate and Behaviour – November 2015).

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Smyth DV *et al*. (1997a). ZA 1296: Toxicity to the Freshwater Diatom, *Navicula pelliculosa*. Brixham Environmental Laboratory, Zeneca Limited, Brixham, UK ; Report No: BL5780/B ; KCA 8.2.6.2/01.

Smyth DV *et al*. (1997b). Chronic toxicity of mesotrione to *Lemna gibba*; Brixham Environmental Laboratory, Zeneca Limited, Brixham, UK Report No: BL5849/B, KCA 8.2.7 / 01.

Vehling, H (2005) Boiling point. Syngenta Crop Protection AG, Basel, Switzerland. Syngenta - Process Hazards Section, Huddersfield, United Kingdom, HT05/135

Zeneca Central Toxicology Laboratory (1994c) E1296: An evaluation in the in vitro cytogenetic assay in human lymphocytes. Report No. CTL/P/4187

Zeneca Central Toxicology Laboratory (1994d) E1296: Assessment of Mutagenic Potential Using L5178Y Mouse Lymphoma Cells. Report No. CTL/P/4183

8 ANNEX I

The following annex has been provided by industry.

REVIEW OF THE MODE OF ACTION (MOA) OF MESOTRIONE IN RODENTS AND ITS RELEVANCE TO HUMAN RISK ASSESSMENT USING THE HUMAN FRAMEWORK ANALYSIS

The following analysis of the mesotrione data-base is based on the methodology developed by an ILSI-HESI workgroup and on the decision logic outlined by Seed *et al* (2005). The human relevance framework (HRF) is based on a four-part analysis:

- Is the weight of evidence sufficient to establish the MoA in animals?
- Are the key events in the animal MoA plausible in humans?
- Taking into account kinetic and dynamic factors, is the animal MoA plausible in humans?
- Statement of confidence; analysis; implications

1. Is the weight of evidence sufficient to establish the MoA in animals?

Key Event 1: Inhibition of HPPD

Mesotrione is a triketone herbicide and exerts its MoA via inhibition of the enzyme HPPD (Lee *et al.*, 1997). HPPD occurs in plants and animals, the 52 active site amino acid residues being similar across phyla and highly conserved within mammalian species.

Table 8-1: HPPD amino acid sequence comparisons across phyla.

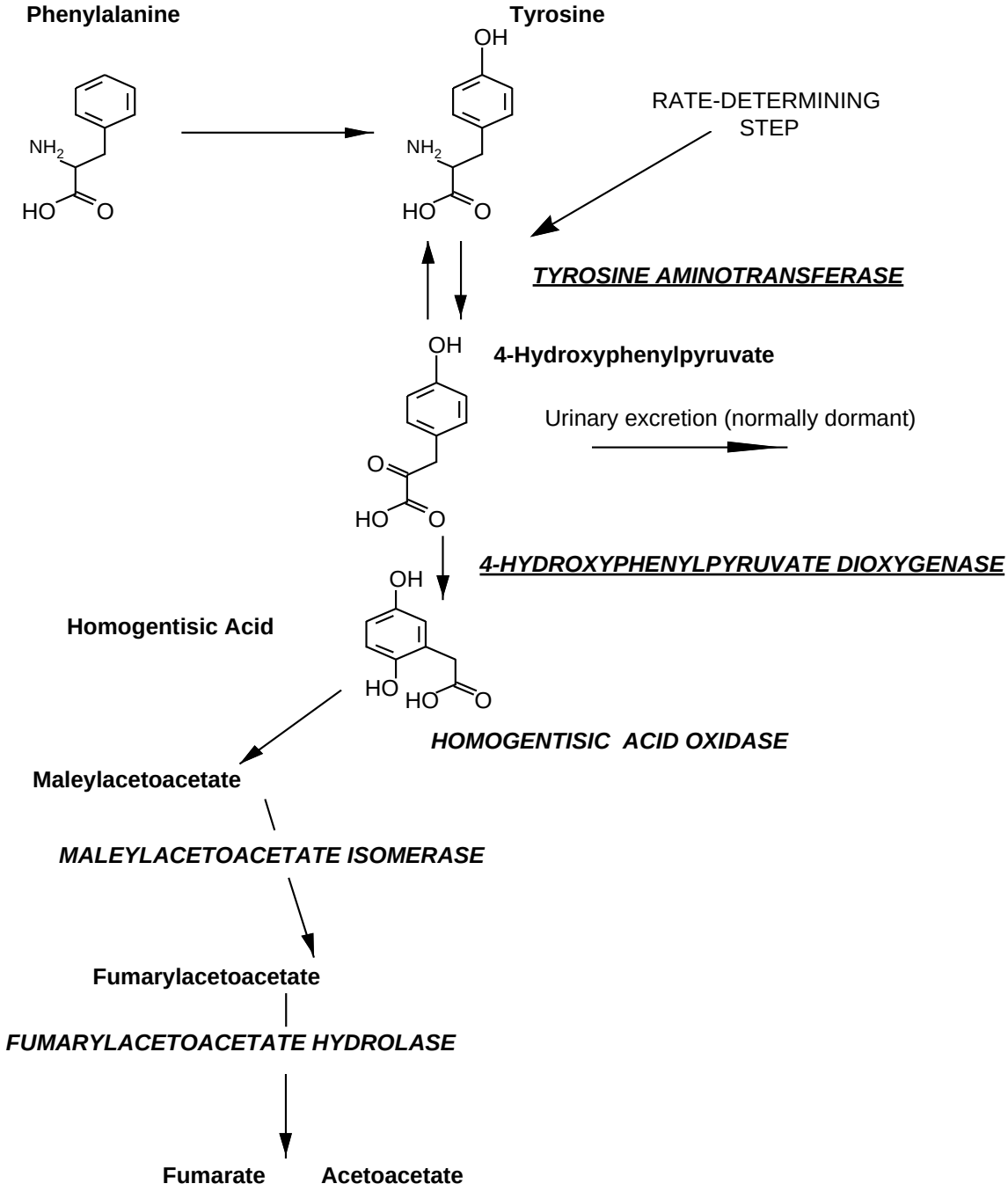
	Arabidopisis	**Maize**	**Rat**	**Mouse**	**Pig**	**Human**
Arabidopisis		60%[1]	32%	31%	30%	32%
Maize	**6**[2]		31%	30%	29%	31%
Rat	**13**	**13**		96%	77%	90%
Mouse	**14**	**13**	**0**		77%	90%
Pig	**14**	**14**	**1**	**1**		
Human	**14**	**13**	**1**	**1**	**2**	

[1]% numbers are the overall % sequence similarity.
[2]Bold numbers are the number of differing active site residues (out of 52 total). From (Yang, et al., 2004)

It can be concluded that mesotrione will inhibit HPPD in both plants and animals and this is supported by direct measurement of hepatic and renal enzyme activity in rats and mice which confirm that mesotrione inhibits HPPD in both species and that this inhibition is reversible (Lock *et al*., 1994).

In mammals, HPPD is the second enzyme in the catabolic cascade of tyrosine (Figure 8-1).

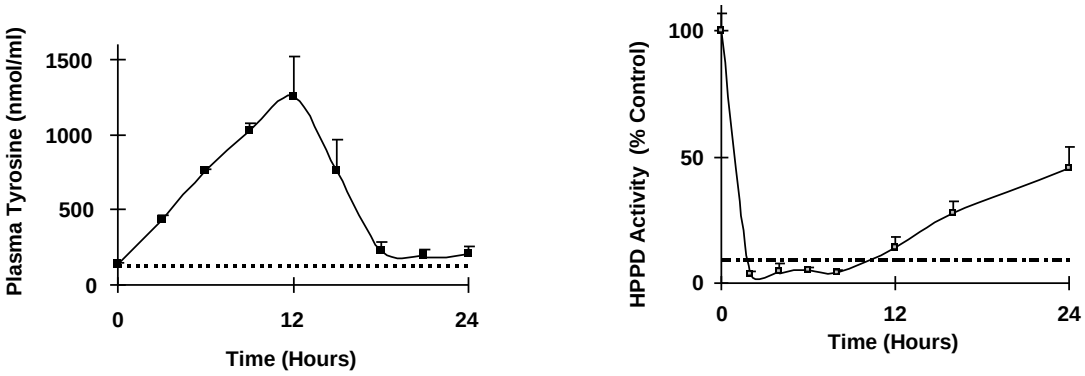
Figure 8-1: Catabolic pathway of tyrosine



Key Event 2: Increase in plasma tyrosine concentration

The consequence of HPPD inhibition is a dose- and species-dependent elevation in plasma tyrosine. If enzyme binding is reversible (as is the case with mesotrione) enzyme activity will recover and plasma tyrosine levels fall once exposure to mesotrione ceases. After a single gavage dose of 2 mg/kg mesotrione to rats enzyme activity starts to recover after 8 hours (Figure 8-2).

Figure 8-2: The 24-hour plasma tyrosine concentration and HPPD activity in male rats after a 2 mg/kg body weight single oral gavage dose of mesotrione.



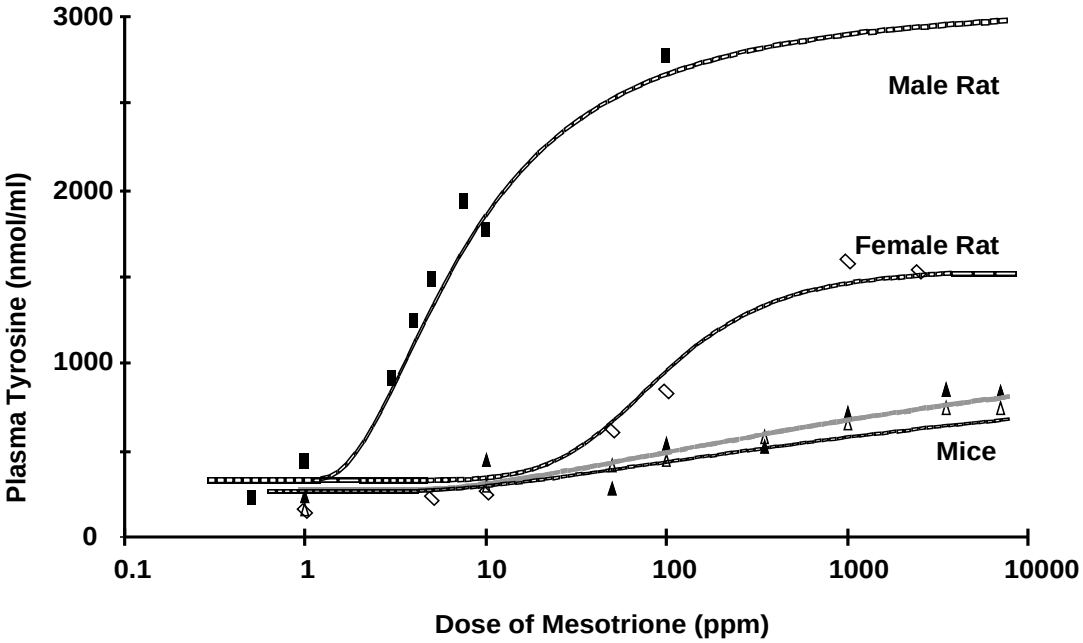
Note 1: dotted line for plasma tyrosine = the mean value in control rats.
Note 2: broken line for HPPD activity = the limit of quantitation of the assay used.
Values on each graph represent the mean plus standard deviation, where n=5.
Reference Lewis RW and Botham JW (2013) A review of the mode of toxicity and relevance to humans of the triketone herbicide 2-(4-methylsulfonyl-2-nitrobenzoyl)1-3-cyclohexanedione. Crit Rev Toxicol 43(3) 185-199

Key Event 3: Clearance of excess tyrosine

Inhibition of HPPD leads to a build-up of its substrate 4-hydroxypheylpyruvic acid (4-HPPA), also known as 4-hydroxyphenylpyruvate, which is found in urine (Ellis *et al.*, 1995). The formation of HPPA from tyrosine by tyrosine aminotransferase (TAT) is reversible and a build-up of HPPA results in an elevation of tyrosine in the plasma (tyrosinaemia). Tyrosine aminotransferase (TAT), the first enzyme in the catabolic pathway, is the limiting and controlling enzyme of tyrosine catabolism. HPPD normally operates at a fraction of its maximum velocity (Lock *et al.*, 1996) and tyrosine concentration is a function of the rate of formation/absorption of tyrosine, the activity of TAT and the efficiency of HPPA elimination by the kidney.

Innate hepatic TAT activity is higher in the mouse and a sex difference in the rat results in TAT activity in the female rat being higher than the male. The difference in TAT activity results in a species and sex difference in tyrosine accumulation (Figure 8-3).

Figure 8-3: Mesotrione induced tyrosinaemia: dose response in male and female rats and mice



References:
Anonymous, (1997d), Anonymous (1997e) and Anonymous (1997j).

Key Event 4: Tyrosine-related spectrum of toxicological effects

In standard regulatory studies a range of toxicities has been seen in rats. Some of these toxicities are seen in mice but only at very high dose levels

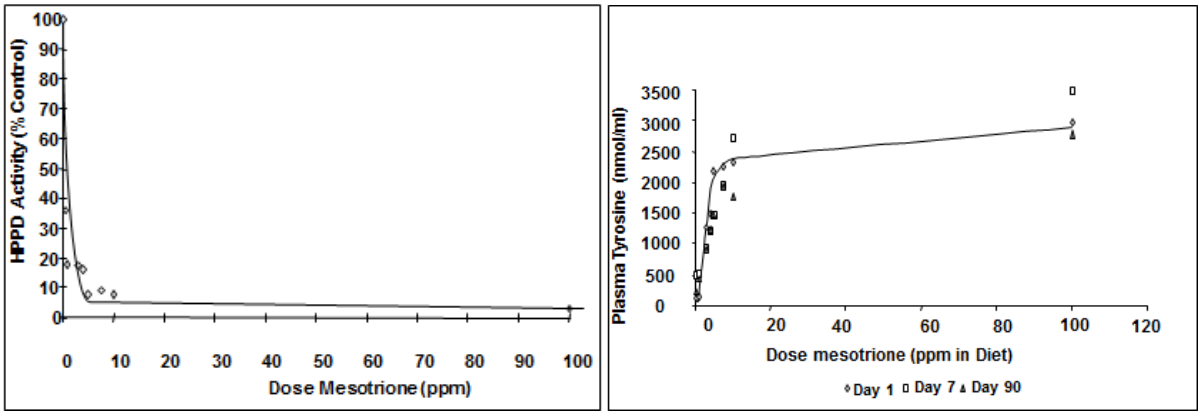
Table 8-2: Toxicities seen in the rat and mouse following mesotrione administration

Effect	Rat	Mouse
Corneal opacity	+	-
Thyroid proliferation	+	-
Sciatic demyelination	+	-
Glomerulonephropathy	+	-
Liver weight increase	+	+/-
Kidney weight increase	+	+/-
Body weight decrease	+	+/-
Reproductive Effects - Litter effects (reduced survival) - Bilateral hydronephrosis	 + +	 - -
Minor modulation in the rate of normal ossification	+	-

i. Dose-response relationships

The relationship of dose of mesotrione administered in the diet to the resultant concentration of tyrosine in the plasma is illustrated in Figure 8-3. This correlates with the inhibition of HPPD as illustrated below (Figure 8-4):

Figure 8-4: HPPD inhibition (a) and Plasma tyrosine concentration (b) in male rats – 90 day administration

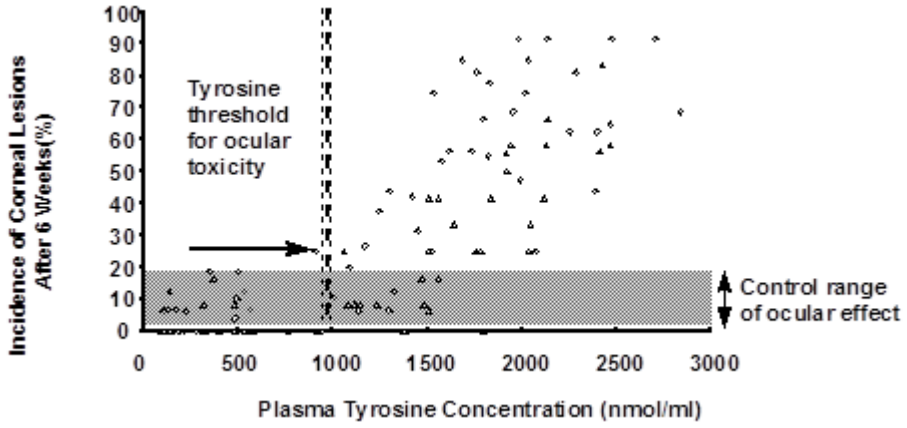


Data taken from studies conducted according to OECD test Guideline 408

It has been shown (Burns *et al*., 1976; Rich *at al*., 1973) that feeding rats on low protein/high tyrosine diets causes corneal opacity identical to that seen in rats administered mesotrione in diets for at least 2 weeks.

Data taken from a large series of studies in which groups of rats were dosed for 6 weeks with different triketone HPPD inhibitor structures showed that there was a linear relationship between plasma tyrosine concentration and ocular toxicity. This study also demonstrated that there was a threshold plasma concentration (approx. 1000 nmol/mL) below which tyrosine-induced corneal leasions do not occur.

Figure 8-5: Relationship of plasma tyrosine concentration to corneal lesions in rats.



An analysis of the dose-response relationship between tyrosine and all adverse effects seen in subchronic and chronic studies in rats has shown that all adverse effects described in the systemic toxicity studies in the rat correlate closely with plasma tyrosine concentration, providing good but indirect evidence that tyrosine is the causal agent of these toxicities, rather than mesotrione itself.

Further details of the characteristic toxicities seen in the rat which have been shown to be associated with elevated plasma tyrosine (tyrosinaemia) are given in Appendix 1.

ii. Strength, consistency and specificity of association

More direct evidence supporting the correlation of tyrosine concentration with toxicological effect is available from exacerbation studies and by examining the species difference in toxicity of mesotrione.

Studies where HPPD is inhibited by mesotrione and the resulting tyrosinaemia is exacerbated by adding excess tyrosine to the diet have been conducted in pregnant rats to evaluate the effect of tyrosine on pregnancy and in rabbits to investigate the effect of tyrosine on fetal ossification. These studies are discussed in Appendix 2. They show a consistency in the direct role of tyrosine as the cause of developmental and reproductive effects in the rat and rabbit and strengthen the evidence that excessive plasma tyrosine is responsible for the toxicity observed.

The spectrum of toxicities seen in rats following administration of mesotrione is different from that seen in mice administered similar doses and/or dosed orders of magnitude higher than rats. The difference is concluded to be entirely attributable to the significantly higher plasma tyrosine concentrations seen in rats (Table 8-3).

Table 8-3: Toxicities and Plasma Tyrosine Concentrations Seen in the Rat and Mouse Following Mesotrione Administration

	Rat			**Mouse**		
Effect	**Presence**	**Plasma tyrosine* (nMole/mL)**	**Mesotrione dose (mg/kg/day)**	**Presence**	**Plasma tyrosine * (nMole/mL)**	**Mesotrione dose (mg/kg/day)**
Corneal opacity	+	> 1000	0.16	-	≈ 800	> 1000
Thyroid proliferation	+	> 1000	< 0.48	-	≈ 800	> 1000
Sciatic demyelination	+	> 1000	< 0.48	-	≈ 800	> 1000
Glomerulonephropathy	+	> 1000	< 0.48	-	≈ 800	> 1000
Liver weight increase	+	≈ 800-1000	< 0.48	+/-	≈ 800	> 1000
Kidney weight increase	+	≈ 800-1000	< 0.48	+/-	≈ 800	> 1000
Body weight decrease	+	≈ 800-1000	< 0.48	+/-	≈ 800	> 1000
Reproductive Effects -Litter effects -Bilateral hydronephrosis	 + +	 ≈ 800-1000 ≈ 800-1000	 1.2 0.3	 - -	 ≈ 800 ≈ 800	 >1000 >1000
Ossification effects	+	≈ 800-1000	< 100	?-	≈ 800	> 600

* Measured or extrapolated from research data

iii. Temporal associations and reversibility

The time dependency of the relationship between elevated plasma tyrosine and genesis of an adverse biological event is demonstrated by the data on ocular opacity. A sustained plasma tyrosine

elevation in excess of a threshold of approximately 1000 nmol/mL plasma needs to be maintained for 6 weeks to reliably induce ocular change. Most of the pathological findings associated with mesotrione exposure, such as sciatic nerve demyelination, thyroid proliferation and degenerative kidney change, are only seen following exposures longer than 1 year. Furthermore, effects noted in the rat after mesotrione dosing for 90 days are reversible within four weeks after cessation of dosing. This reversal coincides with clearance of mesotrione, cessation of HPPD inhibition, and return of tyrosine plasma concentrations to control group levels.

It can be concluded that all early effects occurring with repeated doses of mesotrione are reversible and all chronic effects are seen only when severe tyrosinaemia is sustained for longer than 1 year.

Table 8-4: Reversibility of tyrosine-induced toxicity in the rat: values at end and 4 weeks after termination of a 90-day study conducted to OECD test guideline 409

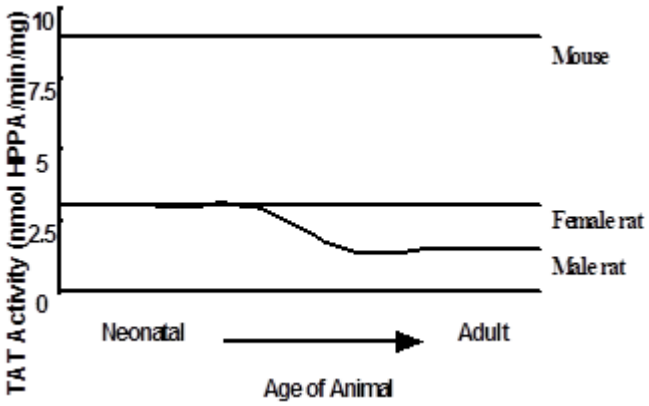
Dietary mesotrione conc.	**5 ppm**		**100ppm**		**2500ppm**	
Week:	**0**	**4**	**0**	**4**	**0**	**4**
HPPD activity (% controls)	11	55	3.5	62	3.8	N/D
Plasma tyrosine conc (% control)	828	101	1382	106	1039	374
TAT activity (% control)	114	104	119	102	166	N/D
Body weight (% difference from control)	-3	0	-2.5	+2	-9	-8
Liver wt/body wt (% control)	110	101	113	107	120	113
Kidney wt/body wt (% control)	112	100	112	99	110.5	110
Ocular effects:						
NAD/total examined	67/80	15/16	24/80	8/16	28/76	6/16
Opacity	10	0	56	0	46	0
Vascularisation	2	0	44	0	41	0
Ghost vessels	0	0	0	0	0	10

Key: Week 0 = week 13/14 of dosing (90 days)

iv. Life stage sensitivity

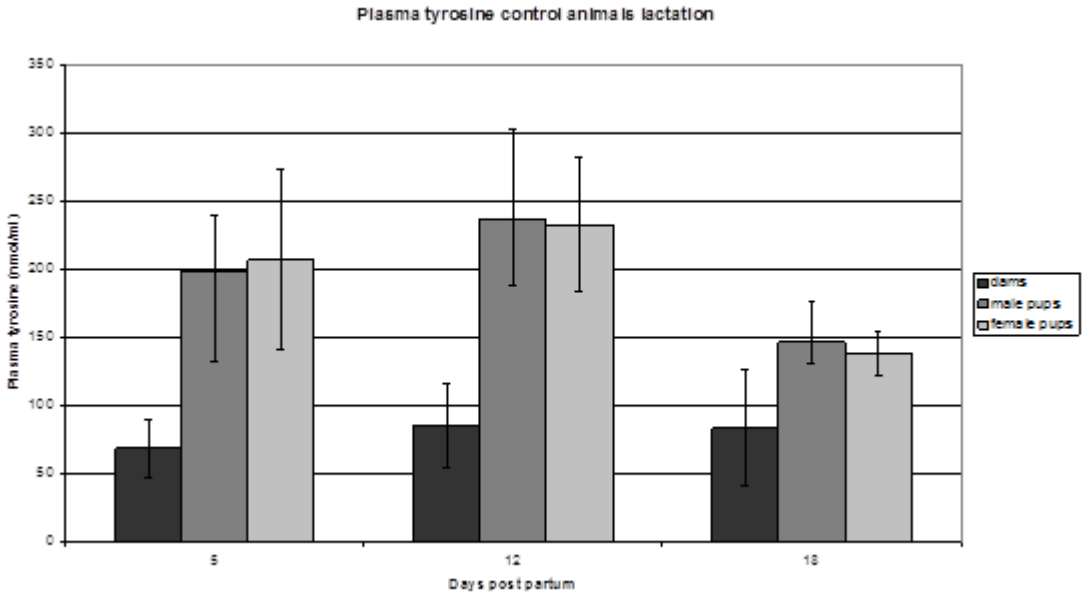
Data are available to demonstrate that neonatal rats and mice are not more sensitive to the inhibition of HPPD than adults. Hepatic TAT activity was measured in untreated rats and mice from day 1 *post partum* to 6 weeks of age. TAT expression at birth is as high as or higher than adult levels. However, in the case of rats (but not mice) between birth and puberty, the TAT levels of male rats falls whereas that of the females remains constant (Figure 8-6).

Figure 8-6: Expression of TAT in rats and mice with age



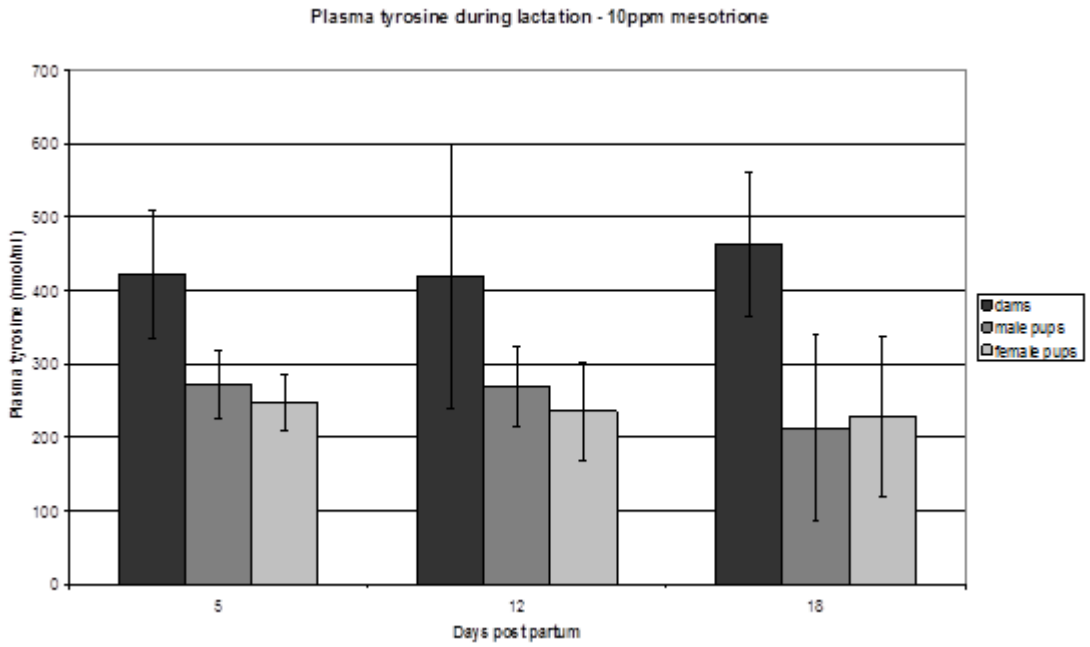
Despite this consistency in TAT expression, the plasma concentration of tyrosine in control mice is higher in pups than in adults.

Figure 8-7: Plasma tyrosine concentration in control animals during lactation



Nevertheless, it has also been shown that elevation of plasma tyrosine, as a consequence of exposure to mesotrione is lower in mouse pups than in the maternal animal.

Figure 8-8: Plasma tyrosine concentration in animals given 10 ppm mesotrione in diet during lactation



From these data it can be concluded that plasma tyrosine concentrations in control pups are higher than those in adults. The addition of 10 ppm mesotrione to diet results in a significant elevation of plasma tyrosine in adults, although average levels do not exceed 500 nmol/mL. On the other hand plasma tyrosine in pups from the 10 ppm mesotrione group is not significantly elevated above age-matched control levels during lactation and remains < 500 nmol/mL. These data demonstrate that there is no evidence for increased sensitivity of neonatal animals to the effects of mesotrione.

v. Biological plausibility and coherence of the database

A remarkable consistency is observed in the incidence of plasma tyrosine concentrations in excess of 1000 nmol/mL and the occurrence of adverse effects (ocular change, liver and kidney weight increase, reduced body weight and exacerbation of a range of spontaneous pathology).

In the assessment of early life-stage toxicity in the developmental and reproductive database, the same marked association between the biological endpoints of minor changes in ossification and reduced pup survival and elevated plasma tyrosine has been demonstrated (see Appendix 2).

There is also consistency between the systemic and reproductive toxicity databases where the same elevated liver and kidney weight, reduced body weight and ocular change is again observed in the presence of elevations in plasma tyrosine.

The key events in the animal MoA are summarised below:

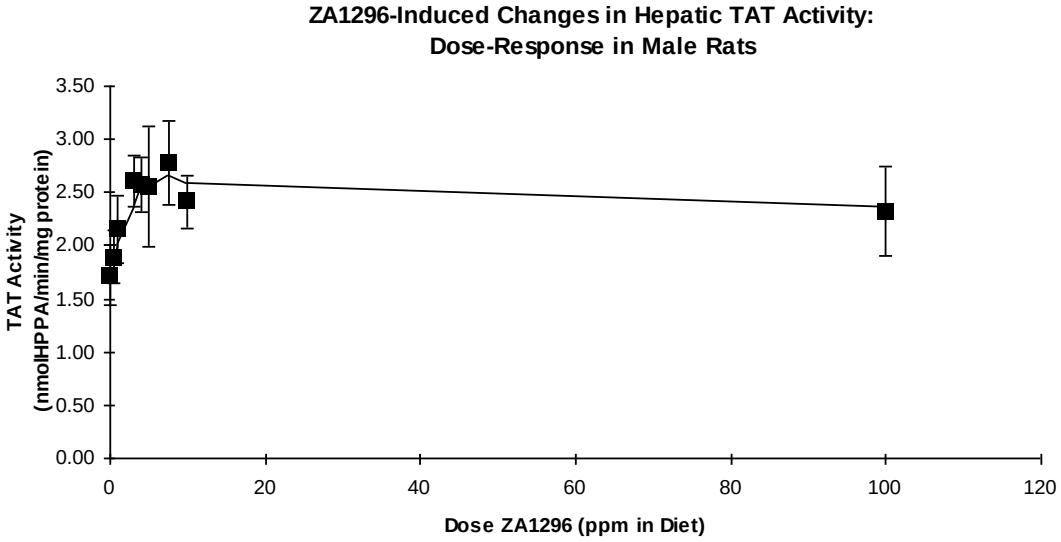
Table 8-5: Key events in the animal MoA

Key Event	Evidence	Reference
Inhibition of HPPD	YES: This is the established herbicidal mode of action in plants. • Has been shown to be the MoA for HPDD inhibitors in mammals • HPPD inhibitors are structurally diverse, but have a common substructure which binds tightly to ➢ a single, common active site ➢ in both plant and mammalian HPPD. • HPPD active site sequence similar ➢ Across plants and animals ➢ Highly conserved across mammalian species.	Lee DL *et al* (1997). Lock EA *et al* (1994)
Increase in systemic tyrosine concentrations	YES: Increases in plasma tyrosine are mesotrione dose-dependent in rat, mouse, and man • Degree of tyrosinaemia is species specific and dependent upon the innate activity of tyrosine aminotransferase (TAT), the rate limiting enzyme in the tyrosine catabolic pathway	Nixon D (2001)
Clearance of excess tyrosine via tyrosine amino transferase (TAT)	YES: TAT is the first enzyme in the catabolic pathway for tyrosine. If the second enzyme (HPPD) is inhibited, excess tyrosine is cleared as phenolic acids in the urine. Rate of clearance depends on inherent activity of TAT which is species dependent – higher in the mouse that the rat. (Mouse > 2x female rat/4x male rat)	Nixon D (2001)
Tyrosine-related spectrum of toxicological effects	YES: Mesotrione administration results in effects on body weight, liver, kidney, eyes, thyroid in subchronic/chronic rat studies and on reproduction/development in the rat. These effects are attributable to severe tyrosinaemia based on: ➢ direct causation of effect by tyrosine alone (ocular opacity) ➢ correlation of effect with tyrosine levels – chronic effect on body weight, liver and kidney, sciatic nerve and thyroid ➢ exacerbation – effect of mesotrione and excess dietary tyrosine on fetal ossification and reproduction	Rich *et al* (1973), Burns *et al* (1976)

vi. Alternate MoAs

Given the strength and consistency of the correlation of tyrosine levels to various toxicological outcomes, the obvious species-dependent generation of high plasma tyrosine levels and the clear dose response to tyrosine rather than mesotrione it is unlikely that there are alternative MoAs. No other MoAs have been identified for mesotrione that are able to unite the range of biological effects observed. Mesotrione does not inhibit tyrosine aminotransferase (TAT), the first enzyme in the tyrosine catabolic cascade.

Figure 8-9: Mesotrione induced changes in hepatic TAT activity – male rats



NMR analysis of urine from rats dosed with mesotrione shows excess phenolic acids (Lock *et al*., 1996), which is consistent with inhibition of HPPD. There was no evidence from urinary analysis of excess levels of tyrosine itself, tyramine nor n-acetyltyrosine, which would have indicated TAT inhibition, nor fumarylacetoacetate, maleylacetoacetate or homogentisate, which would have indicated an inhibition of fumarylacetoacetate hydroxylase, maleylacetoacetate isomerase or homogentisic acid oxidase. In addition, mesotrione does not inhibit tyrosine-hydroxylase, a key enzyme in the anabolic pathway of tyrosine. Therefore, mesotrione has been shown to inhibit a single enzyme (HPPD) in the catabolic pathway of tyrosine and to not affect the key enzyme in the anabolic pathway.

There are some other MoAs that are known to operate with other HPPD inhibitors such as hepatic P450 induction. The lack of significant P450 induction by mesotrione was confirmed by the modest increase in liver weight in chronic studies, the lack of pathological changes in the liver and by a short term study (Anonymous, 1997x)[1] showing that the relevant enzymes were not induced.

vii. Conclusions—assessment of postulated MoA in animals and statement of confidence

Overall there is a high level of confidence in the postulated MoA that tyrosine elevation leads to a range of biological effects, which are consistent with those seen predominantly in rats following mesotrione exposure. This confidence is based on evidence for direct causation between plasma tyrosine and ocular change, where ocular tyrosine concentrations and effects in the eye are highly correlated. Furthermore, there is a large and convincing database showing a consistent positive correlation between all the biological endpoints described following mesotrione treatment and elevations in plasma tyrosine concentration. Additionally, tyrosine exacerbation studies have established a firm link between the severity of developmental and reproductive changes and the degree of plasma tyrosine elevation.

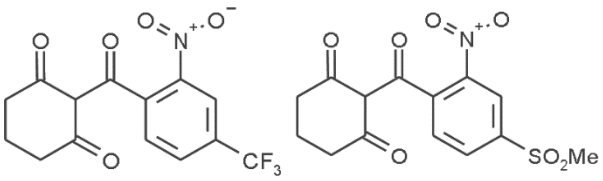
It is concluded that the weight of evidence is sufficient to establish a MoA in animals.

[1] See study summary on page 157 (end of Annex I)

2. Are the key events in the animal MoA plausible in humans?

i. HPPD inhibition and increase in tyrosine

Humans have HPPD that is similar to both rat and mouse HPPD and, in particular, the amino acid sequence homology in the active site is nearly identical, differing by only one amino acid residue. Nitisinone (also known as NTBC and Orfadin®) is an HPPD inhibitor (Lock *et al.*, 1994) that is structurally similar to mesotrione and is used therapeutically to treat humans with Tyrosinaemia Type 1 (an autosomal recessive fumarylacetoacetate hydrolase deficiency).



Nitisinone (NTBC) Mesotrione

Human data are available from volunteer studies, clinical trials and over 2000 patient years of experience which demonstrate that HPPD inhibitors (nitisinone and mesotrione) cause an increase in plasma tyrosine concentration (Lindstedt *et al.*, 1992; Hall *et al.*, 2001). Therefore, based on HPPD enzyme similarities between humans and rodents and based on studies with HPPD inhibitors, it is considered that mesotrione is able to inhibit human HPPD and can increase plasma tyrosine concentrations.

ii. Clearance of tyrosine via TAT

The level of TAT activity in humans is similar to that of male mice, and they are thus able to efficiently catabolize tyrosine.

Table 8-6: Comparison of innate hepatic TAT (nmol/ HPPA/min/mg protein) activity – rats, mice and humans

	Rat	Mouse	Humans*
Male	1.7 ± 0.2	7.8 ± 1.5	7.17 ± 1.17
Female	3.3 ± 0.5	10.5 ± 1.9	

* From Henderson *et al*., 1981

As is the case for mice and rats, in humans TAT is fully active 24 hours after birth (Krechmer, 1959).

iii. Tyrosine-related spectrum of toxicological effects

There are limited reliable data on toxicity in humans occurring as a consequence of elevated plasma tyrosine concentrations. In patients treated with nitisinone it is reported that eye disorders, including conjunctivitis, photophobia, eye pain, keratitis and corneal lesions have been noted, some of which were transient and/or reappeared (FDA, 2002) although none occurred in adults treated for alkaptonuria (Sunwannarat *et al.*, 2005). Although alkaptonuria patients were recorded with plasma tyrosine concentrations which periodically reached 800 nmol/mL none showed treatment related

ocular effects. However, there are no data from patients with plasma tyrosine concentrations that significantly exceed these concentrations for prolonged periods.

Nonetheless, there is no evidence to suggest that humans would react differently than rats to high, sustained levels of tyrosine. For nitisinone it is recognized that humans could exhibit adverse effects should tyrosine levels reach toxicologically relevant concentrations and FDA therefore recommended that plasma tyrosine levels should be kept below 500 µmol/L in order to avoid toxic effects caused by high plasma tyrosine levels, i.e., corneal lesions and hyperkeratotic lesions and neurological symptoms. In summary, the key events seen predominantly in rats are plausible in humans, although there are significant species differences in the level of plasma tyrosine that can accumulate.

3. Taking into account kinetic and dynamic factors, is the animal moa plausible in humans?

The mode of action for mesotrione-related adverse effects seen predominantly in rats depends on the sustained elevation of tyrosine. Two factors determine whether or not humans would sustain high plasma tyrosine levels, firstly residence time of the HPPD inhibitor in the body (basic kinetics) and secondly the efficiency of clearance of tyrosine by TAT.

Table 8-7: Key events in the animal MoA and human relevance

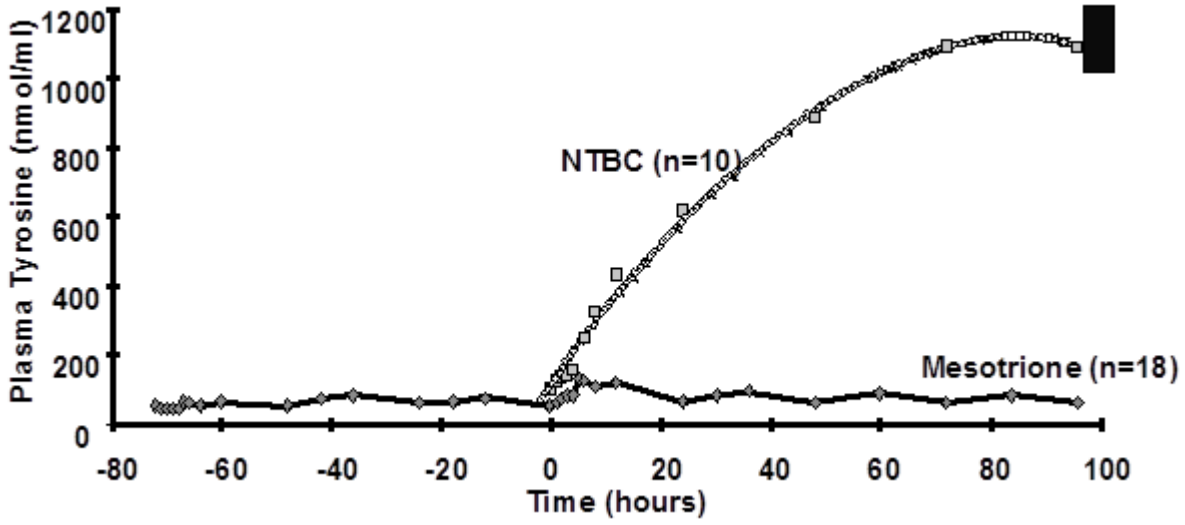
Key Event	Evidence in Rats	Evidence in Mice	Evidence in Humans	References
Inhibition of HPPD	YES: Mesotrione – measured in rats	YES: Mesotrione - measured in mice	YES: Indirect data from HT1 patients treated with nitisinone	Lindstedt *et al* (1992)
Increase in systemic tyrosine concentrations	YES: Dose-dependent increase in plasma tyrosine concentration	YES: Dose-dependent increase in plasma tyrosine concentration	YES: Dose-dependent increase in plasma tyrosine concentration – from mesotrione HVS and nitisinone trials	
Clearance of excess tyrosine via tyrosine amino transferase (TAT)	YES: Tyrosine cleared slowly. TAT activity[1] ➢1.7 (males) ➢3.3 (females) Maximum plasma tyrosine concentration (nmoles/mL). ➢2500-3000 (males) ➢1500-2000 (females)	YES: TAT activity[1] ➢ 7.8 (males) ➢10.5 (females) Maximum plasma tyrosine conc. is 800 nmoles/mL	YES: TAT activity[1] ➢7.17 Plasma tyrosine max concentration similar to mouse when HPPD completely inhibited.	Henderson *et al* (1981) Hall *et al* (2001)
Tyrosine-related spectrum of toxicological effects	Ocular toxicity evident when plasma tyrosine concentration >1000 nmol/mL Chronic effects and reproductive/ developmental effects at	Minimal effects on liver and kidney weight seen in mouse studies – plasma tyrosine < 1000 nmol/mL	No adverse effects seen in human volunteer study with mesotrione or in healthy volunteers with nitisinone. Nitisinone showed minor effects in patients if plasma tyrosine exceeds 800-1000 nmol/ml Nitisinone has no adverse effects in adults treated for	Hall *et al (*2001) FDA (2002) Sunwannarat *et al*

	similar plasma tyrosine concentration		alkaptonuria where plasma tyrosine reaches 600-700 nmol/mL.	(2005)

Kinetics

Experience with nitisinone, a potent HPPD inhibitor used as a drug in children for the treatment of Tyrosinaemia Type 1 (HT1) shows that elevated plasma tyrosine levels can be achieved with this drug. Limited data for mesotrione indicate that comparable doses do not cause significantly elevated tyrosine levels in human volunteer studies (Figure 8-10) although higher doses (4 mg/kg) can produce minor transient elevations in plasma tyrosine (Hall *et al.*, 2001).

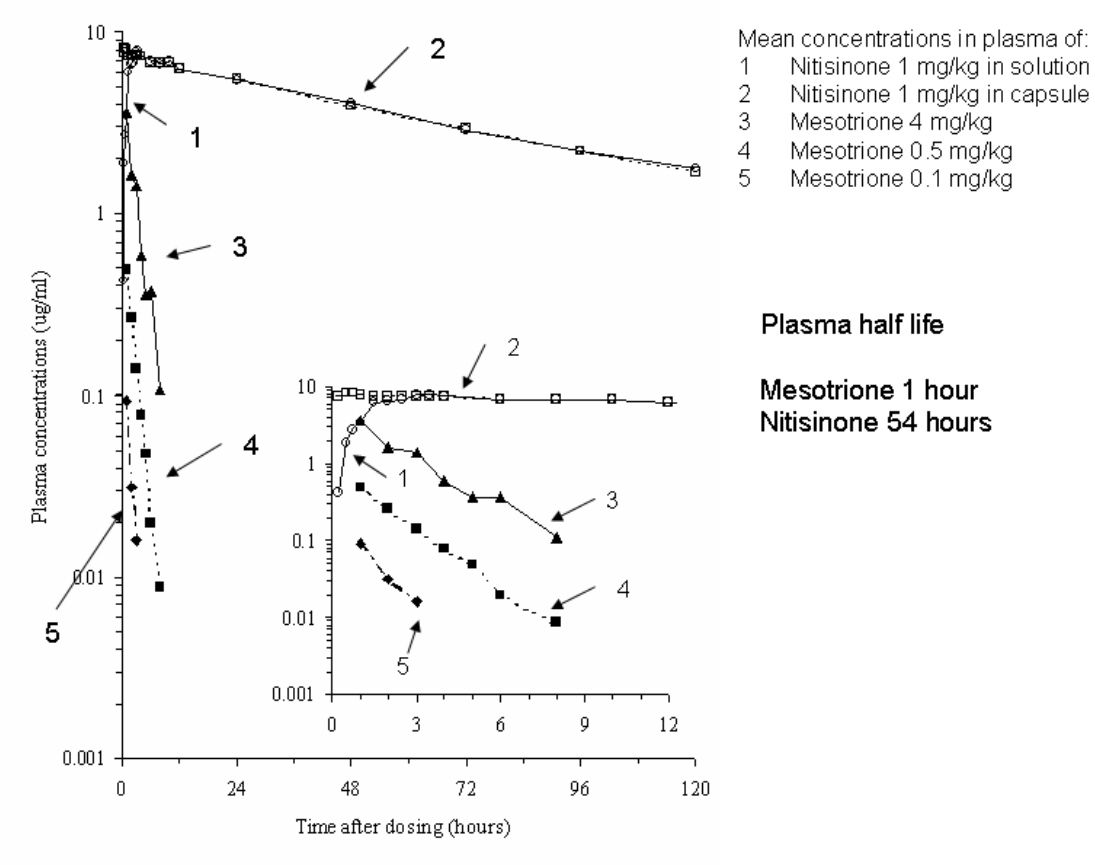
Figure 8-10: Plasma tyrosine levels in humans after nitisinone or mesotrione dosing



Nitisinone 1 mg/kg Mesotrione 0.5 mg/kg

The reason for the difference between nitisinone and mesotrione plasma tyrosine concentrations is that nitisinone has a plasma half-life of 54 hours compared to mesotrione's half-life of 1 hour (Figure 8-11), resulting in a plasma area-under-the-curve (AUC) for nitisinone that is 400x greater than mesotrione. The sustained presence of nitisinone results in elevated tyrosine levels that plateau at about 1000 nmol/mL.

Figure 8-11: Clearance of nitisinone or mesotrione from human volunteers

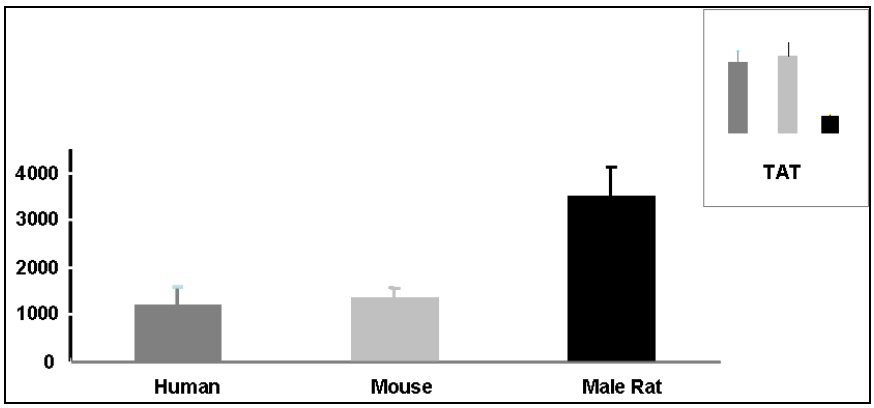


Therefore, the kinetics of an HPPD inhibitor is important in understanding whether or not the MoA would, in reality, occur in humans. Mesotrione's short half-life would suggest that the MoA would not result in adverse findings in humans unless very high repetitive doses were administered to elevate tyrosine and for a sustained period of time.

Clearance of tyrosine

The toxicities attributable to mesotrione have been shown to be significantly different in the two rodent species studied in detail, rats and mice (Table 2). The metabolic fate of mesotrione has been studied following single and repeat doses in rats and mice and it has been demonstrated that there are no species differences in the metabolism and excretion of mesotrione which could explain the species difference in toxicity reported (Gledhill *et al*., 2001). The differences in toxicity profile are attributable to differences in the steady-state plasma tyrosine concentration under conditions of complete HPPD inhibition, which in turn has been shown to be dependent upon the animals' innate TAT activity. Human TAT activity is much higher than the rat and thus at equivalent doses of the potent HPPD inhibitor nitisinone plasma tyrosine concentrations in mice and humans are much lower than those seen in the rat.

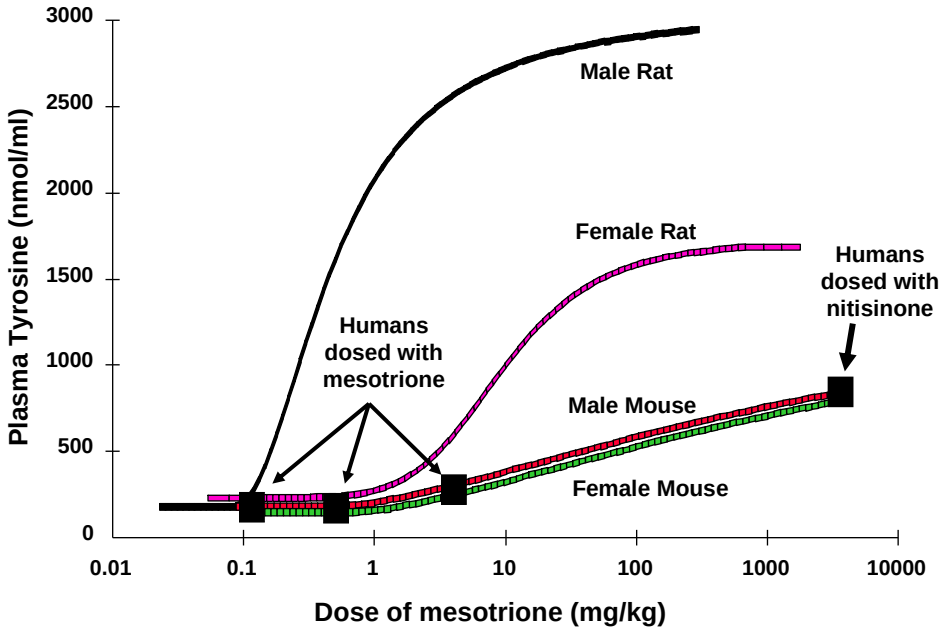
Figure 8-12: Nitisinone-induced plasma tyrosine (nmol/mL) elevation in rats, mice and humans



Nitisinone dose = 1 mg/kg
Human data from clinical trial in 10 male volunteers (Hall, 2001)
Data for rat from Lock *et al.*, 1996. Data for mouse from Lock *et al.*, 2000.

Using values taken from the human volunteer study with mesotrione and the clinical trial with nitisinone (Hall *et al*., 2001) and assuming a 400-1000-fold difference in potency of nitisinone and mesotrione, it is possible to extrapolate steady-state plasma tyrosine concentrations in humans following mesotrione exposure. These data points have been added to the graph produced for mice and rats and show that humans achieve tyrosine levels similar to mice and that these do not exceed 1000 nmol/mL (the threshold established for induction of adverse effects seen in the rat).

Figure 8-13: Mesotrione induced tyrosinaemia in mouse, rat and humans: Relationship between plasma tyrosine concentrations and dose of



Mesotrione

On the basis of these data, which clearly indicate that humans will not achieve the high tyrosine levels seen in the rat, the effects of mesotrione in man would be expected to be the same as those seen in the mouse and to be significantly different from those seen in the rat.

In summary, the kinetic and dynamic characteristics of mesotrione in humans are such that the short half-life (kinetics) and the efficient removal of tyrosine (dynamics) obviate the likelihood of any tyrosine-related effects that are seen in the rat after mesotrione dosing. Therefore, taking into account kinetic and dynamic factors, the animal MoA is plausible in humans but with the practical certainty that adverse effects will not be observed in humans.

4. Conclusion and statement of confidence

Based on the available data, the mode of action and the key events for mesotrione-related adverse effects have been identified and, on a qualitative basis, are plausible in humans. Given the quantitative factors (kinetics and dynamics) of this mode of action (short half-life and the significant differences in TAT activity between humans and rats), humans are unlikely to exhibit the toxicities seen in rats. Nonetheless, mesotrione at some relatively high dose level may raise tyrosine levels in humans but certainly not to an extent or for a duration that is likely to cause adverse effects.

In addition the available data lead to the conclusion that for tyrosine-related toxicities the mouse is the most appropriate model to use for the human risk assessment of mesotrione.

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Report:	Anonymous (1997x) Biochemical studies in rat and mouse liver

Induction of liver enzymes in response to administration of mesotrione were studied in CD rats and CD-1 mice. There was no significant effect of ZA1296 on liver weight in rats or mice. Mesotrione caused small increase in certain CYP enzyme activities: ECOD, EROD, MROD and PROD were increased in rats fed 7000 ppm or higher, BROD was increased in mice fed 3000 ppm and higher and EROD was increased in mice fed 7000 ppm. CYP isoform profiles showed a minimal increase in 1A1 in rats fed ZA1296 and minimal increases in 2B1/2 and 3A1 in mice fed mesotrione.

There were no histopathological change in livers of rats fed mesotrione and in mice; only slight liver hypertrophy was present at 3000 and 7000 ppm. Livers from rats and mice examined by electron microscopy showed proliferation of smooth endoplasmic reticulum but no evidence of peroxisome proliferation in either species.

There was no evidence of hepatoxicity, hepatomegaly or peroxisome proliferation. Mesotrione caused only minimal CYP induction at high dietary levels and is considered unlikely to be hepatocarcinogenic in rats and mice.

APPENDIX 1: KEY TOXICITIES EVALUATED IN MOA STUDIES

Ocular toxicity

Following repeated administration of mesotrione to rats for more than 2 weeks, ocular effects are seen. These are characterised initially by hazy opacity or single or multiple small opacities which are histopathologically keratitis. With continued administration and/or administration of higher doses of mesotrione the extent of the opacity can progress to involve the entire cornea and to include vascularisation. The lesions in rats:

- Are seen at lower dose levels of mesotrione in males than females
- Are reversible, as assessed by ophthalmoscope and histopathology, following cessation of mesotrione administration
- Are seen following administration of tyrosine in low protein diets
- Are related to plasma tyrosine concentrations

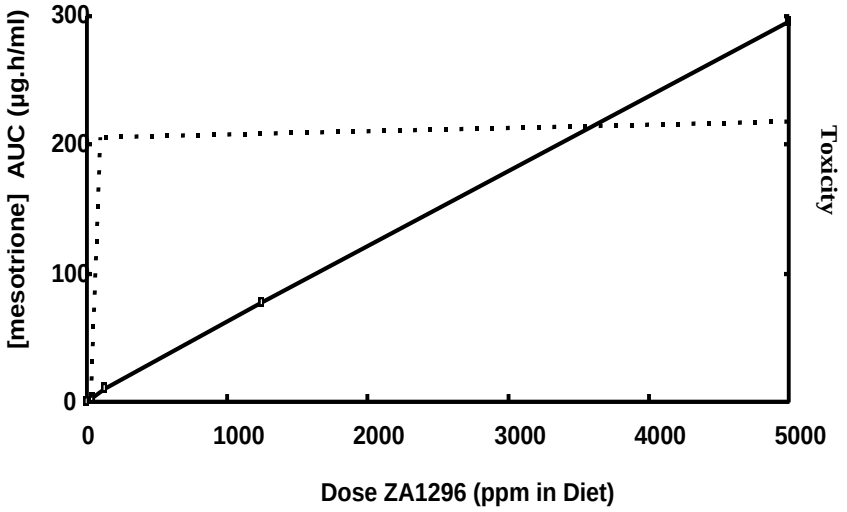
Liver and kidney enlargement

An increase in absolute and relative liver and kidney weight, in the absence of clinical or histopathological change, is also seen following repeat administration of mesotrione. The dose response does not follow the plasma concentrations of mesotrione, being maximal at low doses of mesotrione. The dose response does, however, parallel that of tyrosine concentrations in the plasma which reach maximal levels at very low dietary concentrations of mesotrione and plateau thereafter (Figure 8-13 and Figure A1-1).

As is the case with the ocular effects, the liver and kidney weight increases:

- Are seen at lower dose levels of mesotrione in males than females
- Show evidence of reversibility
- Are clearly related to plasma tyrosine concentration (Figures A1-2/3), the response to plasma tyrosine being linear
- In the case of the liver, have been shown not to be related to peroxisome proliferation or CYP induction

Figure A1-1: Schematic representation of dose-response relationship of bioavailability and toxicity of mesotrione in male rats



Key: ——— Mesotrione levels in plasma

..... Toxicities associated with administration of mesotrione

Figure A1-2: Mesotrione induced liver growth and tyrosinaemia: Dose response in male rats at 90 days

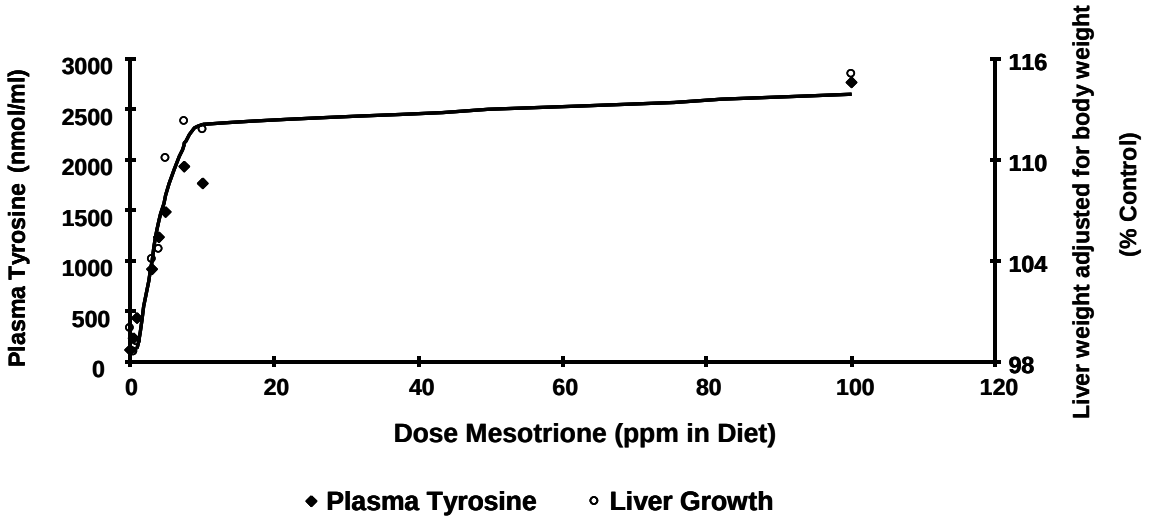
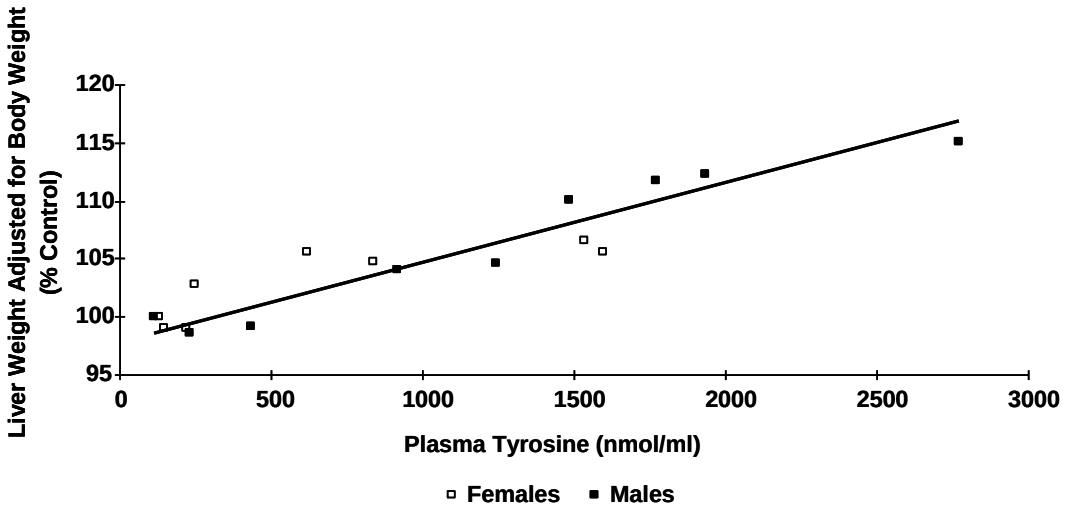


Figure A1-3: Tyrosine-mediated liver growth in rats: Dose response in rats after 90 days dietary administration of mesotrione

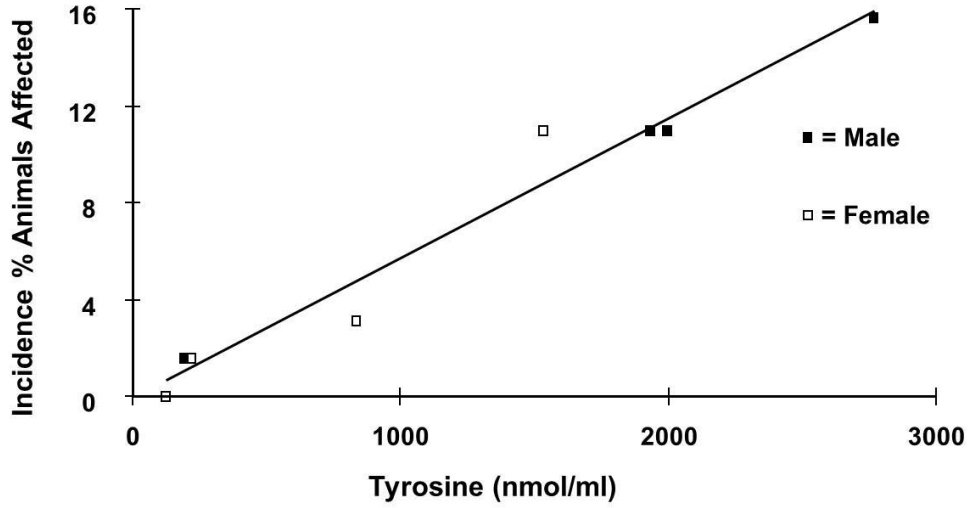


R^2 = 0.881

Chronic toxicities

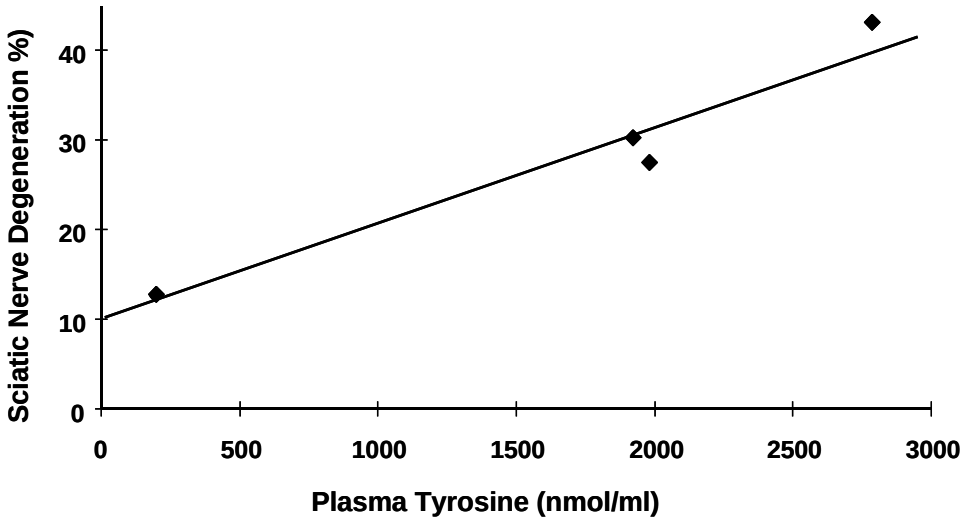
In addition to those effects noted in rats following subchronic administration of mesotrione, toxicities noted in rats after dosing for more than 1 year include an increase in follicular cell proliferative lesions of the thyroid gland and an increased severity of the spontaneous age related lesions of sciatic nerve demyelination (both sexes) and chronic progressive glomerulonephropathy (males). As is the case for ocular toxicity, these findings showed a linear association with plasma tyrosine concentration, rather than administered dose of mesotrione, providing evidence that tyrosine is the causal agent inducing these changes.

Figure A1-4: Relationship between thyroid proliferative lesions and plasma tyrosine concentrations in male and female rats



R^2 = 0.981

Figure A1-5: Relationship between sciatic nerve demyelination (severe form) and plasma tyrosine concentrations in male rats



R^2 = 0.967

In contrast to the range of toxicities seen in the rat, very few changes are seen in the mouse after subchronic or chronic administration, even at limit dose 1000 mg/kg bw/day mesotrione. Apart from a slightly lower body weight and marginally higher organ weights at very high doses no treatment related toxicities are apparent. Specifically, ocular toxicity and changes in spontaneous pathologies are not seen in the mouse.

In the rat there is direct evidence (ocular toxicity) and indirect but supportive evidence (organ weight changes, chronic pathologies) that effects seen in the rat are attributable to severe tyrosinaemia rather than a direct effect of mesotrione administration. These toxicities are either not seen, or seen only at very high dose levels (liver weight increases), in the mouse where plasma tyrosine concentrations, although elevated, are lower than those in the rat. There is no significant difference in the absorption, metabolism, disposition and excretion of mesotrione in rat and mouse (Gledhill *et al.*, 2001). Hence the species difference does not result from a difference in systemic exposure to parent or metabolites. It is concluded that the clear species difference in the toxicity of mesotrione to rats and mice is attributable to differences in systemic exposure to tyrosine.

APPENDIX 2: EXACERBATION STUDIES WITH MESOTRIONE AND TYROSINE

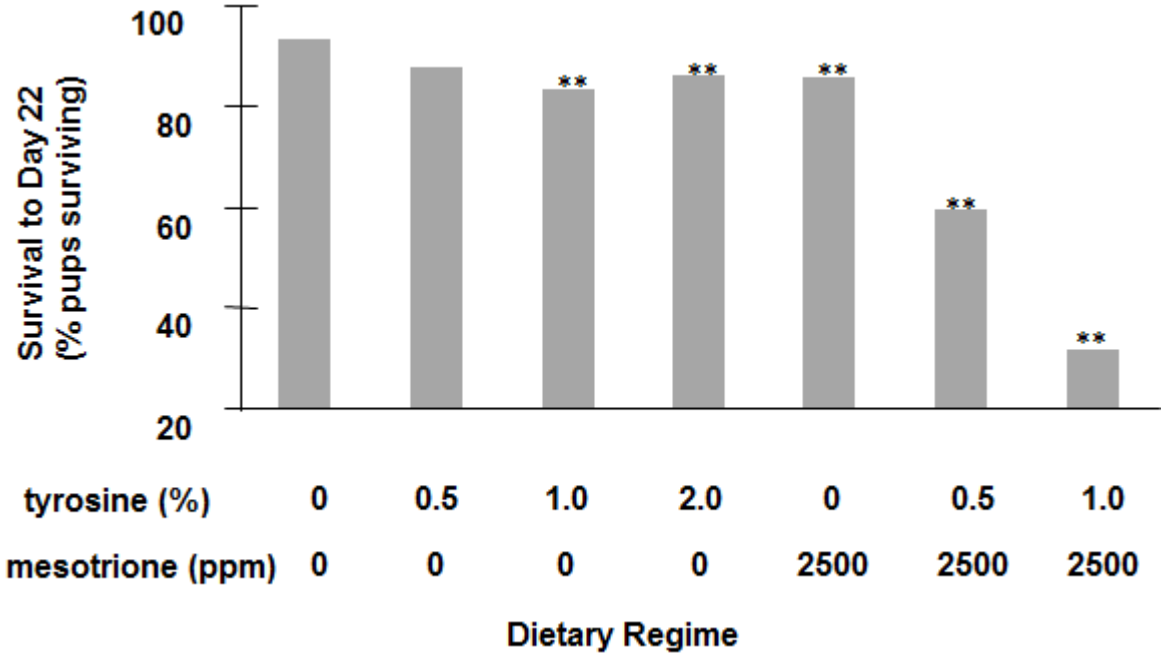
Reproductive Toxicity

In the rat multigeneration study, poor pup survival was noted particularly on days 1-4 *post partum*. In addition an increased incidence of bilateral hydronephrosis was noted in pups.

A study was conducted to investigate the potential for increasingly severe tyrosinaemia to affect pup survival (Anonymous 1997q, Anonymous 2000b; RAR: B.6.8.2.8 – refer to section 4.11.1 of the CLH report). Groups of time mated female rats were fed diets containing increasing levels of tyrosine (0.5%-2%) or diets containing 2500 ppm mesotrione supplemented with increasing levels of tyrosine (0.5%-2%). Excessive toxicity was noted in the group fed diets containing 2500 ppm mesotrione and 2% tyrosine and this group was terminated on gestation day 11. There were minimal effects on maternal or pup body weights in all other groups.

There was a dose related increase in the incidence of whole litter losses in the study and a decrease in litter size on day 1 providing good evidence of a causal relationship between tyrosine and reduced litter size/increased perinatal mortality in the rat (Figure A2-1).

Figure A2-1: Pup survival to day 22 in the investigative reproduction study in rats



** Statistically significant difference from control group mean at the 1% level (Student's t-test, 2-sided)

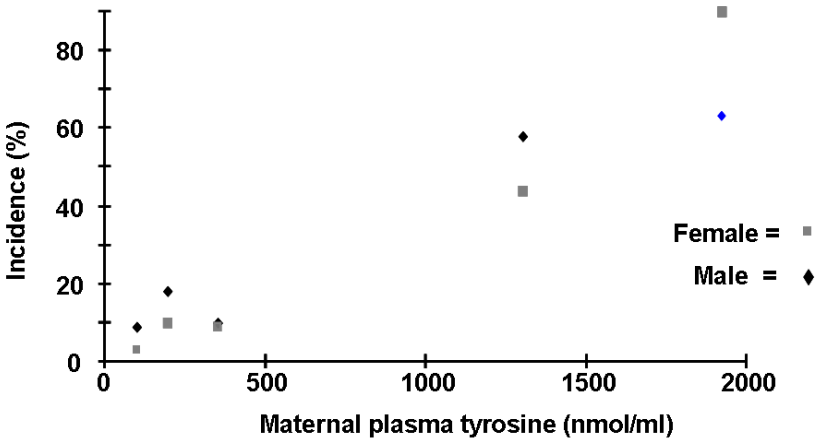
There was an increase in pup death in the tyrosine/mesotrione groups in the early postnatal period. The incidence of whole litter losses was 1, 2, and 2 in the 0.5%, 1% and 2% tyrosine alone groups and 6 and 8 in the 0.5% and 1% tyrosine/mesotrione groups. There was no occurrence of whole litter loss in the control group.

Mesotrione alone resulted in a slight decrease in the percentage of pups born live. 0.5% and 1% tyrosine/mesotrione resulted in a dose-related decrease in the percentage of pups born live. Litter size from day 5 onwards was statistically significantly reduced for both tyrosine/mesotrione groups

and dose-related. Pup survival at day 22 was slightly reduced in the mesotrione alone group and markedly reduced in the 0.5% and 1% tyrosine/mesotrione groups; the majority of pups died before day 5.

This study also evaluated the effect of tyrosine on the incidence of bilateral hydronephrosis which had been noted as a finding in pups from the rat reproduction study (Figure A2-1). The study had not been designed to specifically evaluate this endpoint and an increase in incidence/severity of the lesion in pups from litters administered mesotrione and additional dietary tyrosine was not established. However, a clear relationship to tyrosine concentration in the plasma was noted in the guideline study.

Figure A2-2: Relationship of group mean maternal plasma tyrosine to the incidence of bilateral hydronephrosis in rat pups (Anonymous, 1997p)



In the rat, administration of mesotrione in a 3 generation reproduction study clearly resulted in a variety of effects on pups which are considered to be a consequence of the severe tyrosinaemia seen in rats after inhibition of HPPD. The NOAEL for effects in the rat was 2.5 ppm (0.3 mg/kg bw/day).

Mesotrione had no effect on the same parameters in the mouse at doses up to and including limit dose (7000 ppm). The mouse parental toxicity NOAEL was 1500 ppm based on a slightly lower body weight in F0 males in the premating phase and a slightly increased incidence of lenticular opacity (which is distinct from the tyrosine related corneal opacity) in F1 adults at 7000 ppm. The mouse offspring systemic NOAEL was 350 ppm based on slightly lower body weight compared to controls after pups had started to consume diet. Based on the amount of diet consumed by such young pups this equates to a very conservative NOAEL of 71 mg/kg bw/day.

Developmental Toxicity

Mesotrione was assessed for teratogenic potential in the rat, rabbit and mouse. There was no evidence of teratogenicity in any species. In both the rat and rabbit there was evidence of a clear change in the pattern of skeletal ossification at all dose levels. All changes were identified as variants – minor transient changes in areas undergoing ossification of cartilage to bone immediately prior to birth. None of the alterations in ossification were considered to have any adverse effect on post natal development due to their transient nature as part of an ongoing process.

In order to further evaluate the reason for the changes in ossification pattern seen in both the rat and rabbit prenatal developmental toxicity studies, a study was conducted to evaluate the consequence of increasingly severe tyrosinaemia on ossification of the fetal skeleton in the rabbit.

Groups of time mated rabbits were assigned to groups as described in Table A2-1.

Table A2-1: Assignment of rabbits to groups in the study to investigate the effects of tyrosinaemia on skeletal ossification in the rabbit

Group	Treatment by gavage	Diet
1	Water	Standard diet
2	500 mg ZA 1296/kg bw/day	Standard diet
3	500 mg ZA 1296/kg bw/day	1% tyrosine in standard diet
4	Water	1% tyrosine in standard diet

[Note: In this table the treatment groups are listed in the same number order as was given in the original study report. In the main CLH dossier (Table 18) the groups are described in a differet order but he treatment regimes are the same.]

There were no treatment related visceral effects in this investigative study. An apparent increased incidence in vessels arising from the aortic arch in groups 3 and 4 was concluded to be unrelated to treatment as no such effect had been noted in the study with varying doses of mesotrione (Anonymous, 1999).

The treatment regime resulted in elevated mean plasma tyrosine and a corresponding alteration in ossification. Some examples of the changes in ossification in the spinal column and ribs are illustrated below (Figure A2-3 and Figure A2-4).

Figure A2-3: Relationship between plasma tyrosine concentration and percentage incidence of specific rabbit foetal skeletal ossification changes

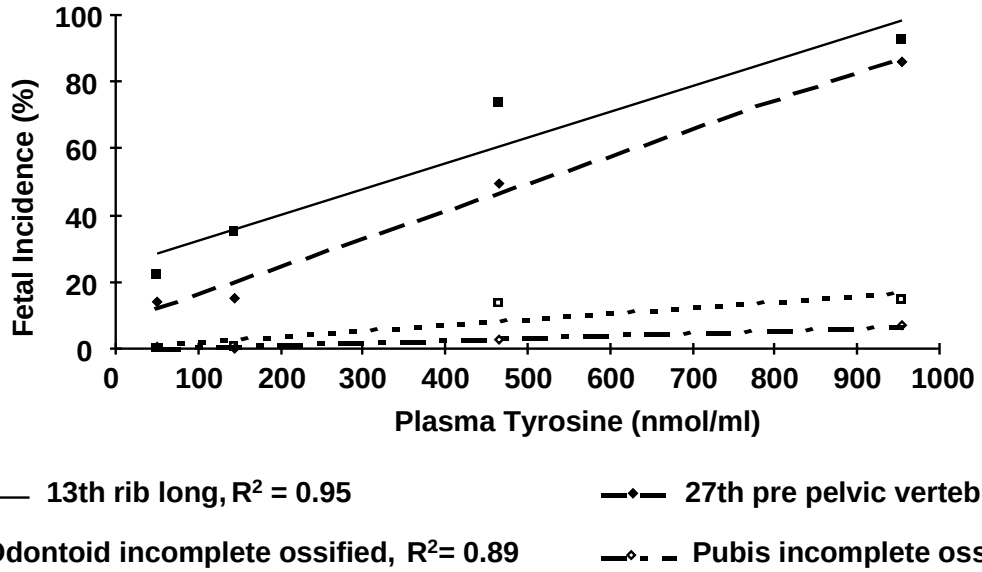
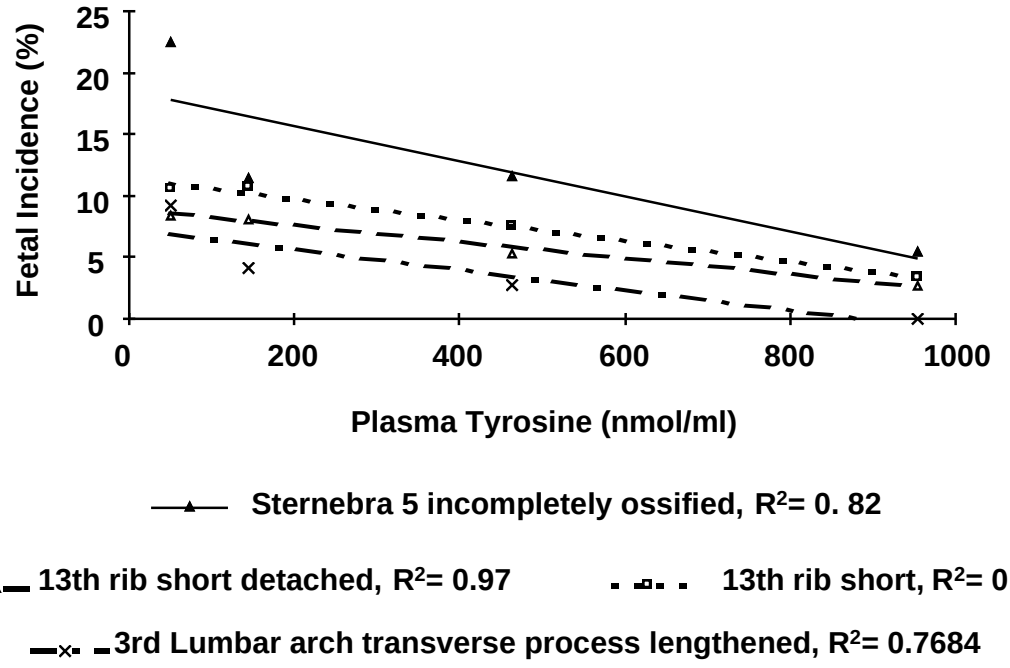


Figure A2-4: Relationship between plasma tyrosine concentration and percentage incidence of specific rabbit foetal skeletal ossification changes



In this study the alterations in foetal ossification, as seen in the main regulatory study, were confirmed. There was a good correlation between the incidence of specific changes in ossification and plasma tyrosine concentrations in the dams, indicating a causal relationship with tyrosine rather than a direct effect of mesotrione on foetal development.

In both the reproductive and developmental toxicity studies there is good evidence of a causal relationship between significantly elevated tyrosine concentrations in the plasma and effects seen in the rat and rabbit.