Mesotrione

NOTIFICATION OF AN ACTIVE SUBSTANCE UNDER COMMISSION REGULATION (EU) 844/2012

DOCUMENT M-CA, Section 5 Supplement

TOXICOLOGICAL AND METABOLISM STUDIES ON THE ACTIVE SUBSTANCE

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[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

Table of Contents

CA 5 TOXICOLOGICAL AND METABOLISM STUDIES ON THE ACTIVE SUBSTANCE

Introduction

This document supports the application for renewal of the regulatory approval of mesotrione under Commission Implementing Regulation (EU) 844/2012 of 18 September 2012. This document reviews the toxicological studies, including additional data and risk assessments, for mesotrione.

Mesotrione was included in Annex I of Council Directive 91/414/EEC (Commission Directive 2003/68/EC of 11 July 2003). This active substance is an approved active substance under Regulation (EC) 1107/2009 (repealing Commission Directive 91/414/EEC) as specified in Commission Implementing Regulation (EU) No. 540/2011 of 25 May 2011.

In accordance with Commission Implementing Regulation (EU) 844/2012, this document summarises new information which are relevant for the renewal of the approval of mesotrione under Regulation (EC) 1107/2009. Where appropriate this document refers to the Commission Implementing Regulation (EU) No. 540/2011 for mesotrione and to the Review Report for mesotrione (SANCO/1416/2001 – Final, 14 April 2003), and in particular the endpoints provided in Appendices I and II thereof.

This document covers data and risk assessments which were not part of the original dossier and which are necessary to reflect changes:

- In requirements under Commission Regulation (EU) No 283/2013, and the associated Annex, which repeals Commission Regulation (EU) No 544/2011 which, under Regulation (EC) 1107/2009, replaced the requirements of Annex II to Directive 91/414/EEC
- In scientific and technical knowledge since the approval or last renewal of the approval
- To representative uses

Where the conclusions of the EU review had specific areas of concern on mesotrione, new data and/or reviews and/or risk assessments have been provided. Where additional and/or new data on mesotrione are provided, a justification has been included. Also a justification has been given if new data are required but none were provided.

Details of the literature search undertaken can be found in M-CA Section 9. If a relevant scientifically peer-reviewed open literature reference has been identified for mesotrione or its major metabolites, it has been discussed within the relevant data point.

CA 5.1 Studies on Absorption, Distribution, Metabolism and Excretion in Mammals

New studies have been completed since the original review of mesotrione (**Mesotrione (ZA1296) DAR, Volume 3, December 1999**). An overview is provided below and detailed summaries are provided in CA 5.1.1. These new data support the existing conclusions and are provided as they better reflect the requirements of the new guidelines, specifically pharmacokinetics and tissue depletion in both rats and mice.

Table 5.1-1: Summary of new ADME studies

Title	Author/Date	Report Number
Mesotrione: Excretion and distribution following a single oral dose (1 mg/kg) in the rat.	Duerden A (2005) Syngenta File No. ZA1296/2022	CTL/UR0828
Mesotrione: Pharmacokinetics and tissue depletion following a single oral dose (1 mg/kg or 100 mg/kg) in the rat.	Duerden A (2005a) Syngenta File No. ZA1296/2018	CTL/UR0836
Mesotrione: Absorption, pharmacokinetics, tissue depletion and excretion study following a single oral dose (1 mg/kg or 100 mg/kg) in the mouse.	Smith A, (2005). Syngenta File No. ZA1296/2195	CTL/UM0827

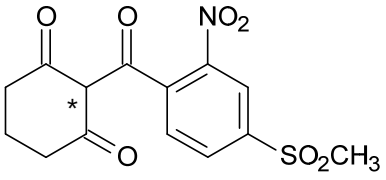
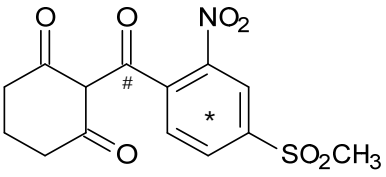
These data are discussed in context with the data previously evaluated in the **Mesotrione (ZA1296) DAR Volume 3, December 1999**.

The mammalian metabolism of mesotrione (ZA1296) 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. In biotransformation studies, the quantitative and qualitative nature of metabolites formed was 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 QWBA study there were no clear differences in tissue distribution profiles between the [^{14}C-dione]- or [^{14}C-aromatic]- mesotrione.

Therefore, in the new ADME studies it was sufficient to use only [^{14}C-aromatic]-radiolabeled material.

The structure of mesotrione and position of the radiolabels is shown below:

Table 5.1-2: Radiolabelled forms of mesotrione used in ADME studies – Structure and position of the label.

Radiolabel	Structure and position of label
[^{14}C-dione]-mesotrione ([Cyclohexane-2-^{14}C]-mesotrione)	 (* = ^{14}C position)
[^{14}C-aromatic]-mesotrione ([Phenyl-U-^{14}C]-mesotrione)	 (* = ^{14}C position) (# = The exocyclic carbonyl group was enriched with carbon-13 for some studies)

Absorption

Systemic absorption of mesotrione in rats following a single oral dose of 1 mg/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 (***Macpherson, D, 1996c***).

The derived absorption values are summarised below; 67% for males and 70% for females (based on the mean values from two studies; ***Macpherson, D, 1996a*** and the new study, ***Duerden A, 2005***) and the mean value for males and females is 69%. 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 (Macpherson, D, 1996b). Very similar absorption values for both doses and sexes were obtained.

Table 5.1-3: Oral absorption of mesotrione in rat

	Studies							
	Oral;1 mg/kg (Macpherson, D, 1996a)		Oral; 1 mg/kg (Deurden A, 2005a)		Intravenous; 1 mg/kg (Macpherson, D, 1996b)		Oral; 100 mg/kg (Macpherson, D, 1996b)	
	Male	Female	Male	Female	Male	Female	Male	Female
Total radioactivity in urine and cage-wash* (% dose)	59.8	61.9	51.2	59.6	82.4	87.2	67.4**	69.4**
Absorption value %	72.6	71.0	62.2	68.3	n/a	n/a	67.4	69.4

* These values have been corrected for total recovery of administered radioactivity.
** These values are the sum of radioactivity in urine, cage-wash and tissues and have been corrected for total recovery of administered radioactivity

Oral absorption of mesotrione in mice was calculated from the sum of radioactivity measured in urine, cage-wash and tissues after correction for the recovery of administered radioactivity. This was estimated as 54% for males and 76% for females following a single oral dose of 1 mg/kg (mean values from the two studies ***Gledhill 1997*** and the new study, ***Smith 2005***) and 70% for males and 74% for females following a single oral dose of 100 mg/kg. The value calculated for 1 mg/kg males was considered to be an underestimate due to sampling (see study summary) so in this case a mean oral absorption of mesotrione in mice of 69% was calculated using the data from both dose groups.

Thus overall, taking account of the new data provided, similar absorption of mesotrione of *ca.*70% was seen in both rats and mice.

Excretion

Most (over 70%) of the radioactivity was eliminated within the first 24 hours after dosing for male and female rats administered a single oral dose of 1 or 100 mg [^{14}C]-mesotrione/kg bw. Renal elimination represented the principal route of excretion of radioactivity independent of sex, dose or dose route accounting over 50% of the total corrected for recovery (including cage wash). At the termination of the studies the total amounts of radioactivity excreted in urine and faeces for both male and female rats were 80-93% 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.

A similar excretion profile was observed in the mouse with much (over 85%) 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 the termination of the excretion studies, 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 residues in expired air were below the limit of detection.

Distribution

Whole body autoradiography after 24 hours showed the greatest intensity of radiolabelling was present in the contents of the gastrointestinal tract, which was consistent with the observed faecal excretion of radioactivity. Radiolabelled residues, for both the labelled forms of mesotrione, were apparent in both sexes in the liver and kidney. No significant residues were observed in any other tissues. At 48 hours after dosing no marked differences in the relative intensity of radioactivity in tissues was seen between the sexes.

Three days after single oral dosing, the highest tissue concentrations in both sexes were present in the liver and kidneys at the termination of the excretion and distribution studies. For rats administered 1 mg mesotrione/kg (***Macpherson, 1996a***), liver contained 12 and 9% (1.846 and 1.748 µg/g) and kidneys 0.3 and 0.9% (0.281 and 0.979 µg/g) of the dose for males and females, respectively which together accounted for over 90% of the radioactivity present in the carcass. Similar results of 9 and 7% (1.39 and 1.43 µg/g) in liver and 0.2 and 0.7% (0.19 and 0.88 µg/g) in kidneys of for males and females, respectively were observed in the new oral dosing study (***Duerden, 2005***) and closely matched those obtained from rats following a similar single intravenous dose of 1 mg mesotrione/kg. After 100 mg mesotrione/kg, liver contained 0.2% (3.529 and 3.655 µg/g), for both sexes, and kidneys 0.008 and 0.013% (0.281 and 0.979 µg/g) 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. The renal concentrations were higher in females than in males for both doses. With the exception of liver and kidneys, all other tissue residues were negligible. 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 (***Lock et al., 2000***[1]). Furthermore, comparisons of the results from the different doses used in the studies suggest that the capacity for liver to retain mesotrione is saturable.

[1] Lock E *et al* (2000). Tissue distribution of 2-(2-nitro-4-triflouromethylbenzoyl)-cyclohexane-1,3-dione (NTBC) and its effects on enzymes involved in tyrosine catabolism in the mouse. *Toxicology* **144** (200) 179-187

Pharmacokinetics

Following a single oral dose of 1 or 100 mg[^{14}C]-mesotrione/kg bw there was little difference between the pharmacokinetic parameters calculated for blood for male and female rats. Maximum blood concentrations (C_{max}) were observed 0.5 hours (t_{max}) after a dose of 1 mg/kg with apparent C_{max} concentrations of 0.27 and 0.25 µg equivs/g for males and females, respectively and 1.5 hours after a dose of 100 mg/kg with apparent C_{max} concentrations of 40.4 and 19.9 µg equivs/g. The half-lives ($T_{1/2}$) were 1.6 and 1.4 hours in males and females respectively for the lower dose and 1.7 and 1.8 hours for the higher dose. This corresponds to the observed elimination profile where most of the absorbed radioactivity is excreted in the early sampling time points (0-6 and 6-12 h).

Table 5.1-4: Pharmacokinetic parameters following oral administration of mesotrione to rat

Parameter	1 mg/kg		100 mg/kg	
	Male	Female	Male	Female
C_{max} (µg equivs/g)	0.27	0.25	40.4	19.9
t_{max} (hours)	0.5	0.5	1.5	1.5
$T_{1/2}$	1.6	1.4	1.7	1.8

The pharmacokinetic profile of mesotrione in mice was similar to that seen in the rat. Following a single oral dose of 1 mg [^{14}C]-mesotrione/kg bw, peak radioactive concentrations (C_{max}) were measured 1 hour after dosing in both sexes (t_{max}) and were 0.06 µg equivs/g in males and 0.08 µg equivs/g in females. Blood concentrations declined in both sexes and reached background values within 24 hours of dosing. There was no difference between the sexes. Following the 100 mg/kg bw dose, the profiles were again similar in both sexes. The peak [^{14}C]-concentrations were observed 1 hour after dosing for both males (5.04 µg equivs/g) and females (14.3 µg equivs/g). Despite the higher initial concentration in female blood, concentrations declined rapidly in both sexes and were below the limit of detection within 6 hours of dosing for both males and females.

Table 5.1-5: Pharmacokinetic parameters following oral administration of mesotrione to mice

Parameter	1 mg/kg		100 mg/kg	
	Male	Female	Male	Female
C_{max} (µg equivs/g)	0.06	0.08	5.0	14.3
t_{max} (hours)	1.0	1.0	1.0	1.0
$T_{1/2}$*	4.2	2.1	0.9	1.0

* Half-life estimate not reported but subsequently calculated using WinNonlin (time points: male 1-12 h , female 2-6 h for the 1 mg/kg dose and 1-4h for male and female for the 100 mg/kg dose

Tissue depletion

Pharmacokinetic data were used to select the time points for the tissue depletion study in rat. The termination times were chosen to be 1, 6, 12, 24, 48 and 96 hours after dosing. For all dose groups, peak tissue concentrations were observed at the first time point measured with the exception of the 1 mg/kg dose female rats where the peak tissue concentration in liver was seen at a 6 hours. The highest tissue concentrations were found in the kidney and liver, for both male and female rats at both dose levels. Blood and plasma residue concentrations were similar over the time course of the experiment. The concentrations in all tissues declined with the levels in many tissues falling below the level of detection before the conclusion of the study. Although residues were still evident in kidney and liver 96 hours after dosing, the levels were seen to decline over time. This is consistent with retention of mesotrione by the liver and the role of these organs in metabolism and excretion. No other discrete tissues typically showed a greater residue than in blood.

All tissues showed a rapid initial rate of elimination (concentrations <10% of peak concentrations within 12-24 h) followed by a slower terminal rate. The rate and extent of the initial tissue depletion meant it was only possible to define a second phase for a limited number of tissues. However, given the extent of the initial decline of radioactivity it is reasonable to assume that this would give a realistic estimate of the half-life driving the kinetics of tissue depletion. For the tissues for which elimination half-lives were calculated, these ranged from 13.8 to 250 hours following a 1 mg/kg dose and 15.8 to 271 hours following a 100 mg/kg dose with the longest calculated for liver and kidney. However, some of the calculated half-lives exceeded the duration of the study and since the concentrations of radioactivity in some tissues, at the latter time points, were close to the limit of detection caution must be exercised when interpreting these data.

The depletion profile of tissue residues for mesotrione in mice was similar to that seen in the rat. Highest tissue concentrations were attained approximately 1 hour after dosing and with no major differences between the sexes at both dose levels. The highest tissue concentrations were found in the kidney and liver, for both male and female rats at both dose levels. The majority of tissue concentrations declined very rapidly, reaching background values within 6 or 24 hours of dosing, irrespective of dose level. Hence, for these tissues, no terminal half-life of elimination could be calculated. In contrast, the elimination of radioactivity from liver and kidneys was slower. After 7 days, irrespective of dose level, the highest residues were present in the liver with lower concentrations in the kidneys and with all other individual tissue concentrations below the limit of detection

Following a dose of 1 mg/kg, the terminal half-lives for residues in the residual carcass were 4.3 days for male mice and 3.7 days for females, with an estimate of 10.5 days for female liver. The observed inter-animal variability for male liver, kidneys and g.i. tract precluded the calculation of reliable half-life estimates for these tissues. Following a dose of 100 mg/kg, the terminal half-life for residues in kidney and the g.i. tract was 3.3 and 3.1 days for male mice and 7.4 and 2.2 days for female mice respectively. The observed inter-animal variability for liver and residual carcass residues made the calculation of half-life estimates unreliable.

^{14}C-Residues were rapidly eliminated from most tissues in rats and mice. Radioactivity in the liver and kidney declined more slowly, attributable to the slower release of radioactivity from tight binding sites in the liver and the role of kidney in excretion. Tissue depletion is consistent with the pharmacokinetic and elimination profiles. The data show that with the exception of the proportion retained by the liver, the absorbed mesotrione was cleared within 12-24 hours of dosing and the capacity of the liver to retain mesotrione was saturable and reversible.

Biotransformation

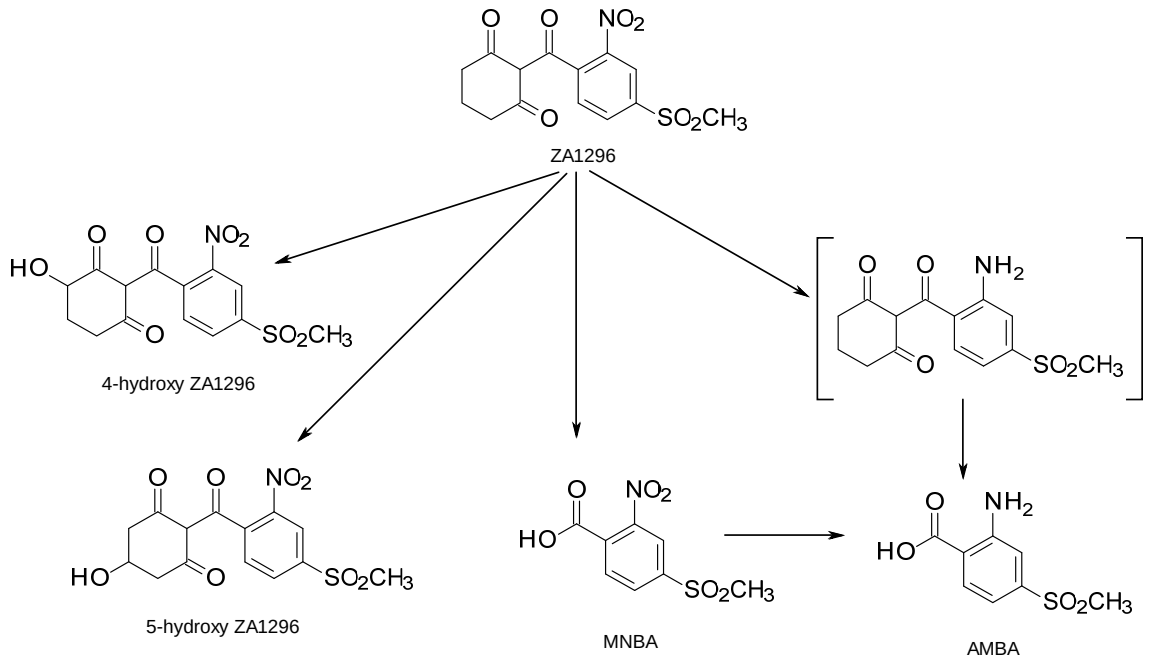
Mesotrione was not extensively metabolised in rats or mice since most of the absorbed dose was excreted unchanged in urine (*ca.* 90% or more of the urinary radioactivity) independent of dose or route. There was a quantitative sex difference apparent in the metabolism of absorbed mesotrione with male rats transforming a higher proportion of parent compound into metabolites than females. Small amounts of minor metabolites including 4-hydroxy mesotrione (this was the principle metabolite being present at 3-5%), 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 hydroxy mesotrione, 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 (Hall 1998) where a large proportion of the dose administered to volunteers was excreted in urine as mesotrione.

In the case of mesotrione a complete ADME database is available in the rat and in the mouse. The data confirm that there is no significant species difference in the way an administered dose of mesotrione is metabolised. In addition data are also available from a human volunteer study (***Hall M, 1997-*** reviewed in the **Mesotrione (ZA1296) DAR December 1999)** and also reported in ***Hall et al 2001***[2]**.** This human volunteer study confirms that metabolism of mesotrione in humans is consistent with that seen in the rat and mouse. It is concluded that data from the appropriate animal models reflect the metabolism of mesotrione in humans.

Biotransformation pathway



Structure in square brackets indicates a postulated intermediate

[2] Hall MG, Wilks MF, McLean Provan W, Eksborg S and Lumholtz B (2001) Pharmacokinetics and pharmacodynamics of NTBC (2-(2-nitro-4-fluoromethylbenzoyl)-1,3-cyclohexanedione) and mesotrione, inhibitors of 4-hydroxyphenyl pyruvate dioxygenase (HPPD) following a single dose to healthy male volunteers. Br J Clin Pharmacol **52**, 169-177 – **referenced in MCA Section 9 for toxicology (relevant literature) and summarised in the Literature Section within CA 5.1. (page 38)**

CA 5.1.1 Absorption, distribution, metabolism and excretion by oral exposure

Report:	KCA 5.1.1/01 Duerden A (2005). Mesotrione: Excretion and distribution following a single oral dose (1 mg/kg) in the rat. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Laboratory Report No. CTL/UR0828/REGULATORY/REPORT, 06 September 2005. Unpublished. (Syngenta File No. ZA1296/2022)

Guidelines

Commission Directive 94/79/EC, No. L 354/18, 51 (1994): US EPA Health Effects Guidelines OPPTS 870.7485: Metabolism and Phramacokinetics (1998): OECD 417 (1984): JMAFF 12 Nohsan No 8147 (2000).

GLP: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

EXECUTIVE SUMMARY

Four male and four female rats were each given a single oral dose of 1 mg [^{14}C]mesotrione/kg bw. The excretion of radioactivity in urine and faeces was monitored for 7 days after dosing. After this period, the rats were killed and residual radioactivity was measured in blood, selected tissues and the remaining carcasses. Additionally, one male and one female rat were each given a single oral dose of 1 mg [^{14}C]-mesotrione/kg bw to investigate the excretion of radioactivity in expired air over 24 hours.

Following a single oral dose of 1 mg [^{14}C]-mesotrione/kg bw, over 85% of the administered dose was eliminated in urine and faeces over 168 hours by male and female rats. Renal excretion in the first 6 hours accounted for over 36% of administered radioactivity in both sexes. The mean total proportion of administered radioactivity excreted in urine was 43% for males and 47% for females, while faecal excretion accounted for 37% and 31% respectively. The percentage of administered radioactivity recovered in the cage wash was 5% for males and 11% for females. The presence of radioactivity in expired air was negligible accounting for less than 0.1% of administered radioactivity over 24 hours. The rates and routes of excretion were similar for males and females.

Seven days after dosing, radioactive residues in the majority of tissues were very low and the tissue distribution of radioactivity was similar for both sexes. The mean total proportion of administered radioactivity present in tissues and carcass was 9% for males and 8% for females. The highest radioactive residue were found in the liver and accounted for approximately 9% of administered radioactivity in males and 7% in females. Residues in kidney were much lower at 0.2% for males and 0.7% for females.

The total recoveries of administered radioactivity were approximately 95% for both males and females.

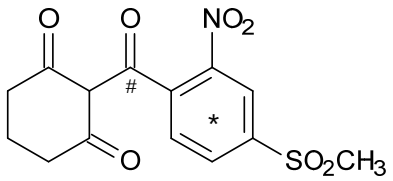
Following a single oral dose of [^{14}C]-mesotrione at 1 mg/kg bw, absorption was rapid with the majority of the administered radioactivity excreted in the first 24 hours in both sexes. The presence of radioactivity in expired air was negligible which is consistent with the known metabolic pathway of mesotrione. The rates and routes of excretion were similar for males and females. Radioactive residues in most tissues were very low with the exception of liver. The tissue distribution of radioactivity was similar for both sexes.

MATERIALS AND METHODS

Materials:

Non-Radiolabelled Test Material:	Mesotrione
Description:	Analytical grade, off white solid
Source:	Syngenta Crop Protection Inc.
Lot/Batch number:	ASW01788-01R
Purity:	99.6% w/w
Stability of test compound:	From information supplied by the Sponsor, the test substance was used within the stated expiry date of January 2005 (stored in a freezer).

Radiolabelled Test Material:	[^{14}C] -aromatic ring-labelled mesotrione
Specific activity:	4.1255 MBq/mg
Radiochemical purity:	99.7 % [determined by TLC and HPLC]
Source:	Syngenta Crop Protection Inc.
Structure:	



* position of [^{14}C]-label; # [^{13}C] enrichment (approx. 40%)

Vehicle: Sodium bicarbonate (10 mg/mL) in distilled water.

Preparation of dosing solutions: Non-radiolabelled and [^{14}C]-radiolabelled mesotrione were dissolved in an aqueous solution of sodium bicarbonate (10 mg/mL) for dosing. The specific activity, homogeneity and stability of the dosing solutions were confirmed by radiochemical analysis.

Test Animals:

Species:	Rat
Strain:	Alpk:APfSD (Wistar-derived)
Age/weight at dosing:	Age not reported / males 225-243 g, females 201-217 g
Source:	Biological Services Section, Alderley Park, Macclesfield, Cheshire, UK
Housing:	Singly in glass (collection of expired air) or stainless steel (remaining animals) metabolism cages
Acclimatisation period:	7 days
Diet:	Rat and mouse No 1 maintenance diet (supplied by Special Diet Services Ltd., Stepfield, Witham, Essex, UK) *ad libitum*
Water:	Mains water *ad libitum*
Environmental conditions:	Temperature: 22±3°C Humidity: 30-70% Air changes: At least 15/h Photoperiod: 12 hours light/12 hours dark

Study Design and Methods:

Experimental dates: Start: 25 June 2004 End: 21 April 2005

Group Arrangements: Animals were assigned by sex to two groups as shown below.

Group	Experiment	Dose (mg/kg bw)	Identities of rats	
			Males	Females
1-2	Low dose expired air	1	1	2
3-4	Low dose excretion balance	1	3-6	7-10

Dosing and sample collection: A single oral bodyweight dependant dose of non-radiolabelled and [^{14}C]-labelled mesotrione dissolved in aqueous sodium bicarbonate solution was administered by gavage to each rat at a dose rate of 4 mL/kg body weight corresponding to a dose level of 1 mg/kg; 4 MBq/kg.

Urine and faeces were separately collected and were frozen immediately upon collection. Exhaled carbon dioxide was collected in traps containing 2N sodium hydroxide and exhaled metabolites were collected in charcoal traps. At the end of the study, the cages were thoroughly washed with sodium bicarbonate solution followed by 1M aqueous hydrochloric acid and the washings retained for radiochemical analysis.

Animals were terminated by exsanguination under terminal anaesthesia. Each terminal blood sample was divided between two heparinised tubes, one of which was centrifuged to separate plasma. The carcasses from animals 1-2 were retained. For groups 2-3, the following tissues were taken for radioactivity measuremnt: adrenals, brain, heart, kidneys, liver, lungs, ovaries, pancreas, spleen, thymus, thyroid, testes, uterus and gastrointestinal tract (contents were retained separately) together with representative samples of abdominal fat (abdominal), bone (femur) and muscle; residual carcasses were retained.

All samples were analysed for radioactivity by liquid scintillation counting either directly or following tissue digestion or sample oxidation.

Sample collection: Animals 1 and 2: Urine was collected at 6 hours after dosing; urine and faeces were collected at 12 and 24 hours after dosing.

Animals 3-10: Urine was collected at 6 hours after dosing; urine and faeces were collected at 12, 24, 36 and 48 hours after dosing and then at 24 hour intervals until the termination of the study. At these times, each cage was rinsed with sodium bicarbonate solution and the washings collected.

Terminal blood samples were collected from all animals by cardiac puncture.

Following termination of each rat, the metabolism cages were washed with sodium bicarbonate solution followed by 1M aqueous hydrochloride and the washings stored prior to analysis.

RESULTS AND DISCUSSION

Dose preparations: The purity, achieved concentrations and homogeneity of the dose preparations were satisfactory.

Excretion of radioactivity: Following a single oral dose of 1 mg [^{14}C]-mesotrione/kg bw, over 85% of the administered dose was eliminated in urine and faeces over 168 hours by male and female rats. Renal excretion in the first 6 hours accounted for over 36% of administered radioactivity in both sexes. The mean total proportion of administered radioactivity excreted in urine was 43% for males and 47% for females, while faecal excretion accounted for 37% and 31% respectively. The percentage of administered radioactivity recovered in the cage wash was 5.2% for males and 10.8% for females. The

presence of radioactivity in expired air was negligible accounting for less than 0.1% of administered radioactivity over 24 hours. The rates and routes of excretion were similar for males and females.

Table 5.1.1-1: **Recovery of radioactivity in excreta after administration of a single oral dose of [^{14}C]-aromatic ring labelled mesotrione to rats (1 mg/kg)**

		Phase 1		Phase 2			
		Percentage of radioactive dose recovered		Percentage of radioactive dose recovered			
		Male *	Female *	Males		Females	
				Mean **	SD **	Mean **	SD **
Urine	0-6 h	34.17	39.94	36.53	8.71	37.77	3.49
	6-12 h	7.48	16.17	5.10	1.37	5.11	1.86
	12-24 h	2.14	5.46	0.94	0.28	1.25	0.43
	24-36 h			0.23	0.13	0.59	0.10
	36-48 h			0.12	0.03	0.42	0.08
	48-72 h			0.11	0.03	0.50	0.17
	72-96 h			0.06	0.01	0.28	0.09
	96-120 h			0.06	0.02	0.29	0.06
	120-144 h			0.05	0.01	0.23	0.02
	144-168 h			0.04	0.01	0.20	0.05
	Subtotal			*43.24*	*9.85*	*46.64*	*5.17*
Faeces	0-12 h	19.82	3.51	23.20	8.34	15.56	6.83
	12-24 h	16.42	6.70	11.37	3.82	11.03	2.03
	24-36 h			1.25	0.58	2.16	1.47
	36-48 h			0.56	0.28	0.74	0.44
	48-72 h			0.17	0.06	0.48	0.11
	72-96 h			0.07	0.01	0.22	0.08
	96-120 h			0.05	0.01	0.20	0.08
	120-144 h			0.10	0.04	0.36	0.07
	144-168 h			0.08	0.04	0.37	0.19
	Subtotal	*36.24*	*10.22*	*36.97*	*12.10*	*31.12*	*10.14*
Cage wash		2.22	10.92	5.15	3.17	10.76	5.41
HCl wash		0.12	0.34	0.01	<0.01	0.06	0.01
Air trap A		0.03	0.03				
Air trap B		0.03	0.03				
Charcoal trap 1		<0.01	<0.01				
Charcoal trap 2		<0.01	<0.01				
Total excreted		82.43	83.11	85.37	0.67	88.58	0.98
Carcass		11.84	10.93				
Tissues and carcass				9.14	0.32	7.82	0.44
Total recovery		**94.27**	**94.04**	**94.51**	**0.86**	**96.40**	**0.67**

* n=1, ** n=4

Tissue distribution of radioactivity: Seven days after dosing, radioactive residues in the majority of tissues were very low and the tissue distribution of radioactivity was similar for both sexes. The mean total proportion of administered radioactivity present in tissues and carcass was 9.1% for males and

7.8% for females. The highest radioactive residue were found in the liver and accounted 8.8% in males and 6.9% in females. Residues in kidney were much lower at 0.2% for males and 0.7% for females.

Total recovery of radioactivity: The total mean percentage recoveries of administered radioactivity including excreta, tissues and residual carcasses following oral gavage dosing at 1.0 mg/kg were 94.5% for males and 96.4% for females.

Table 5.1.1-2: **Tissue distribution of radioactivity after administration of a single oral dose of [^{14}C]-aromatic ring labelled mesotrione to rats (1 mg/kg bw)**

Tissue	Percentage of radioactive dose recovered			
	Males		Females	
	Mean (n=4)	SD	Mean (n=4)	SD
Blood	<0.01	NC	<0.01	NC
Plasma	<0.01	NC	<0.01	NC
Brain	<0.01	NC	<0.01	NC
Abdominal fat	<0.01	NC	<0.01	NC
Heart	<0.01	NC	<0.01	NC
Lungs	<0.01	<0.01	<0.01	NC
Spleen	<0.01	NC	<0.01	NC
Liver	8.83	0.31	6.91	0.44
Kidneys	0.18	0.02	0.73	0.04
GI tract	<0.01	NC	0.01	<0.01
GI contents	<0.01	NC	0.01	0.01
Thyroid	<0.01	NC	<0.01	NC
Thymus	<0.01	NC	<0.01	NC
Ovaries	N/A	N/A	<0.01	NC
Testes	<0.01	NC	N/A	N/A
Pancreas	<0.01	NC	<0.01	NC
Adrenals	<0.01	NC	<0.01	NC
Uterus	N/A	N/A	<0.01	NC
Muscle	<0.01	NC	<0.01	NC
Bone	<0.01	NC	<0.01	NC
Carcass and partial tissues	0.11	0.08	0.15	0.04
Total	**9.14**	**0.32**	**7.82**	**0.44**

N/A - not applicable. NC - Not calculated.

Table 5.1.1-3: **Tissue concentrations of radioactivity after administration of a single oral dose of [^{14}C]-aromatic ring labelled mesotrione to rats (1 mg/kg bw)**

Tissue	µg equivalents of mesotrione/g of tissue			
	Males		Females	
	Mean (n=4)	SD	Mean (n=4)	SD
Blood	<0.01	NC	<0.01	NC
Plasma	<0.01	NC	<0.01	NC
Brain	<0.01	NC	<0.01	NC
Abdominal fat	<0.01	NC	<0.01	NC
Heart	<0.01	NC	<0.01	NC
Lungs	<0.01	<0.01	<0.01	NC
Spleen	<0.01	NC	<0.01	NC
Liver	1.39	0.10	1.43	0.07
Kidneys	0.19	0.02	0.88	0.08
GI tract	<0.01	NC	<0.01	<0.01
GI contents	<0.01	NC	<0.01	<0.01
Thyroid	<0.02	NC	<0.02	NC
Thymus	<0.01	NC	<0.01	NC
Ovaries	N/A	N/A	<0.01	NC
Testes	<0.01	NC	N/A	N/A
Pancreas	<0.01	NC	<0.01	NC
Adrenals	<0.03	NC	<0.01	NC
Uterus	N/A	N/A	<0.01	NC
Muscle	<0.01	NC	<0.01	NC
Bone	<0.01	NC	<0.01	NC
Residual carcass	<0.01	<0.01	<0.01	<0.01

N/A - not applicable.
NC - Not calculated.

Discussion: Following a single oral dose of [^{14}C]-mesotrione at 1 mg/kg bw, over 85% of the administered dose was eliminated in urine and faeces over 168 hours by male and female rats. The majority of the administered radioactivity was excreted in the first 24 hours with over 36% excreted in urine in the first 6 hours in both sexes. This demonstrates that absorption and initial elimination were rapid.

The presence of radioactivity in expired air was negligible which is consistent with the known metabolic pathway of mesotrione. The routes and rates of excretion were similar for males and females. Seven days after dosing, radioactive residues in the majority of tissues were very low and the tissue distribution of radioactivity was similar for both sexes. The highest radioactive residues were found in the liver.

CONCLUSION:

Following a single oral dose of [^{14}C]-mesotrione at 1 mg/kg bw, absorption was rapid with the majority of the administered radioactivity excreted in the first 24 hours in both sexes. The presence of radioactivity in expired air was negligible which is consistent with the known metabolic pathway of mesotrione. The rates and routes of excretion were similar for males and females. Radioactive residues in most tissues were very low with the exception of liver. The tissue distribution of radioactivity was similar for both sexes.

(Duerden A, 2005)

Report:	KCA 5.1.1/02 Duerden A (2005a). Mesotrione: Pharmacokinetics and tissue depletion following a single oral dose (1 mg/kg or 100 mg/kg) in the rat. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Laboratory Report No. CTL/UR0836/REGULATORY/REPORT, 25 August 2005. Unpublished. (Syngenta File No. ZA1296/2018)

Guidelines

Commission Directive 94/79/EC, No. L 354/18, 51 (1994): US EPA OPPTS 870.7485 (1998): OECD 417 (1984): JMAFF 12 Nohsan No 8147 (2000).

GLP: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

EXECUTIVE SUMMARY

In phase 1, nine male and nine female rats were administered a single body weight dependant oral dose of 1 or 100 mg [^{14}C]-mesotrione/kg. Blood samples were taken up to 96 hours after dosing for which the radioactive profiles were measured and pharmacokinetic parameters calculated. In phase 2, 18 male and 18 female rats were dosed similarly and the depletion of radioactivity from a range of tissues, taken over a time course up to 96 hours, was measured.

In the pharmacokinetic experiment the mean peak blood concentration of radioactivity (C_{max}), following a single oral dose of 1 mg [^{14}C]-mesotrione/kg bw to male and female rats, was approximately 0.26 µg equivalents mesotrione/g and occurred 0.5 hours after dosing in both sexes. The half-life was approximately 1.5 hours in both sexes. Following a higher dose of 100 mg/kg bw, C_{max} was 40.4 µg equivs/g in males and 19.9 µg equivs/g in females occurring approximately 1.5 hours after dosing in both sexes. The half-life was 1.7 hours in males and 1.8 hours in females.

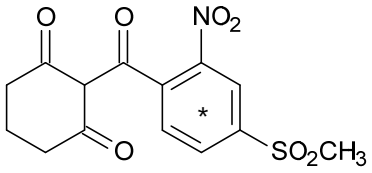
The highest tissue concentrations were found in the kidney and liver, for both sexes at both dose levels. At the 1 mg/kg bw dose level, mean concentrations of mesotrione in the kidney and liver 1 hour after dosing were 3.1 and 2.9 µg equivs/g respectively for males, and 2.0 and 1.6 µg equivs/g respectively for females. These declined to 1.3 µg equivs/g or less by
96 hours after dosing. At the higher dose level, concentrations in kidney and liver 1 hour after dosing were 175 and 48 µg equivs/g respectively for males and 115 and 21 µg equivs/g respectively for females. By 96 hours after dosing the levels had fallen to 1.4 µg equivs/g (kidney) and 2.7 µg equivs/g (liver) or less. At both dose levels the liver and kidney were the only tissues that typically showed greater residues than in plasma. For the animals dosed at 100 mg/kg, higher concentrations were found in the gastrointestinal (GI) tract, but are considered to be attributable in part to GI tract contents.

When comparing males with females, the initial tissue concentrations of radioactivity in females tended to be lower or equivalent to those in males; however at the later time points the concentrations in the majority of tissues were similar in both sexes. It was not possible to determine the elimination half lives for some tissues due to the insufficient definition of the terminal phase of elimination, which for tissues such as brain (1 mg/kg bw dose group) was a consequence of the low residue levels present due to the rapidity with which radioactivity was eliminated. Residues in approximately half of all tissue types taken were below the limit of detection by 96 hours after dosing at both dose levels. For the tissues for which elimination half lives were calculated, these ranged from 13.8 to 250 hours following a 1 mg/kg dose and 15.8 to 271 hours following a 100 mg/kg dose.

Following a single oral dose of either 1 mg or 100 mg of mesotrione/kg bw to both male and female rats, the pharmacokinetic profiles were similar. Residues in the majority of tissues were considered to be low. The highest residues were present in the liver and kidney and were shown to decline slowly over the course of the experiment. No other discrete tissue typically showed a greater residue than in plasma. Residues in approximately half of all tissue types taken were below the limit of detection by 96 hours after dosing at both dose levels.

MATERIALS AND METHODS

Materials:

Non-Radiolabelled Test Material:	Mesotrione
Description:	Analytical grade, off white solid
Source:	Syngenta Crop Protection Inc.
Lot/Batch number:	ASW01788-01R
Purity:	99.6% (w/w)
Stability of test compound:	From information supplied by the Sponsor, the test substance was used within the stated expiry date (31 January 2008) (stored in a freezer)..
Radiolabelled Test Material:	[^{14}C] -aromatic ring-labelled Mesotrione
Specific activity:	4.1255 MBq/mg
Radiochemical purity:	>95 % [determined by TLC and HPLC]
Source:	Syngenta Crop Protection Inc.
Structure:	

* position of [^{14}C]-label

Vehicle: Sodium bicarbonate (10 mg/mL) in distilled water.

Preparation of dosing solutions: Non-radiolabelled and [^{14}C]-radiolabelled mesotrione were dissolved in an aqueous solution of sodium bicarbonate (10 mg/mL) for dosing. The specific activity, homogeneity and stability of the dosing solutions were confirmed by radiochemical analysis.

Test Animals:

Species:	Rat
Strain:	Alpk:APfSD (Wistar derived)
Age/weight at dosing:	Age not reported / males 202-275 g, females 199-238 g
Source:	Biological Services Section, Alderley Park, Macclesfield, Cheshire, UK
Housing:	Up to 5/cage, sexes separately in multiple rat racks suitable for animals of this strain and weight range.
Acclimatisation period:	At least 4 days
Diet:	Rat and mouse No 1 maintenance diet (supplied by Special Diet Services Ltd., Stepfield, Witham, Essex, UK) *ad libitum*
Water:	Mains water *ad libitum*
Environmental conditions:	Temperature: 19-25°C Humidity: 30-70% Air changes: At least 15/hour Photoperiod: 12 hours light/12 hours dark

Study Design and Methods:

Experimental dates: Start: 29 July 2004 End: 28 April 2005

Group Arrangements: Animals were assigned by sex to four groups as shown below.

Phase	Group	Dose (mg/kg bw)	Dose (MBq/kg)	Identities of rats	
				Males	Females
1 (Pharmacokinetic)	1	1	4	1-6, 85-87	7-12, 88-90
	2	100	4	13-18, 91-93	19-24, 94-96
2 (Depletion)	3	1	4	25-39, 97-99	40-54, 100-102
	4	100	4	55-69, 103-105	70-84, 106-108

Dosing and sample collection: A single oral bodyweight dependant dose of the appropriate dosing solution containing [^{14}C]-labelled mesotrione dissolved in aqueous sodium bicarbonate solution was administered by gavage to each rat at a dose rate of 4 mL/kg bw.

Phase 1: Serial blood samples were collected from the tail veins of sub-groups of 3 animals from dose groups 1 and 2. Samples were taken in duplicate. Each venepuncture sample was circa 150 µL and was collected into two 75 µL heparinised haematocrit tubes via a butterfly needle introduced into a tail vein. Terminal blood samples were divided between two pre-weighed heparinised tubes; one was centrifuged to separate plasma.

Phase 2: Sub-groups of 3 animals were terminated at intervals. Terminal blood samples were divided between two pre-weighed heparinised tubes; one was centrifuged to separate plasma. The following whole tissues were removed: adrenals, brain, heart, kidneys, liver, lungs, ovaries, pancreas, spleen, thymus, thyroid, testes, uterus and gastrointestinal tract (contents were discarded) together with representative samples of bone (femur), fat (abdominal) and muscle.

Animals were terminated by exsanguination under terminal anaesthesia. All samples were analysed for radioactivity by liquid scintillation counting either directly or following tissue digestion or sample oxidation.

Sample collection:

Phase 1 blood samples

Group	Animals	Blood collection times (hours after dosing)							
1	1-3, 7-9	1	3	6	10	12	18 (T)		
2	13-15, 19-21								
1	4-6, 10-12	2	4	8	24	30	48	72	96 (T)
2	16-18, 22-24								
1	85-87, 88-90	0.5	1.5 (T)						
2	91-93, 94-96								

(T) - terminal sample

Phase 2 sample collection

Groups	Animal numbers	Termination times (hours after dosing)
3, 4	55-57, 70-72	1
	58-60, 73-75	6
	61-63, 76-78	12
	64-66, 79-81	24
	67-69, 82-84	48
	103-105, 106-108	96

RESULTS AND DISCUSSION

Dose preparations: The purity, achieved concentrations and homogeneity of the dose preparations were satisfactory.

Phase 1 pharmacokinetics: Following a single oral dose of either 1 or 100 mg [^{14}C]-mesotrione/kg, the pharmacokinetic profiles in males and females in each dose group were similar.

The peak concentration of mesotrione (C_{max}), time of peak concentration (T_{max}) and half life for each dose group and sex are tabulated below.

Table 5.1.1-4: Concentrations of radioactivity in whole blood over a time course after administration of [^{14}C]-mesotrione

Sampling time (h)	Group mean blood concentrations (µg equivalents of [^{14}C]-mesotrione/g)			
	1 mg/kg bw		100 mg/kg bw	
	Male (n=3)	Female (n=3)	Male (n=3)	Female (n=3)
0.5	0.265	0.253*	34.664	15.289
1	0.191	0.180*	19.437	11.648
1.5	0.205	0.084	40.408	19.925
2	0.118	0.055	9.800	4.037
3	0.065	0.043	6.929	2.597
4	0.053	0.040	4.231	2.435
6	0.024	0.013*	2.178	1.477
8	0.017	0.012	1.062	1.109
10	0.014	0.008	0.723	0.691
12	0.010	0.007	0.576	0.973
18	0.005	0.005	0.343	0.297
24	0.004	0.013	0.249	1.646
30	0.003	0.003	<0.193	<0.304
48	<0.002	<0.002	<0.156	<0.172
72	<0.001	<0.001	<0.150	<0.185
96	<0.001	<0.001	<0.149	<0.149
C_{max} (µg equiv/g)	0.265	0.253	40.4	19.9
T_{max} (h)	0.5	0.5	1.5	1.5
$t_{1/2}$ (h)	1.6	1.4	1.7	1.8

*n=2

The results obtained during this phase enabled the selection of the termination times for phase 2. The termination times chosen for phase 2 were the same for both dose levels and both sexes, as these times were considered to cover the main points of all four dose groups. These times were 1, 6, 12, 24, 48, 96 hours after dosing.

Phase 2, tissue depletion: The highest tissue concentrations were found in the liver and kidney, for both sexes at both dose levels. Mean concentrations of mesotrione in the kidney and liver of animals dosed 1 mg/kg bw, were 3.07 and 2.92 µg equivs/g respectively for males, and 1.96 and 1.64 µg equivs/g respectively for females, 1 hour after dosing. These declined to 0.27 µg equivs/g (kidney) and 1.30 µg equivs/g for males, and 0.87 and 1.01 µg equivs respectively for females by 96 hours after dosing. At the higher dose level, concentrations in kidney and liver 1 hour after dosing were 175.1 and 47.5 µg equivs/g respectively for males, and 115.3 and 21.0 µg equivs/g respectively for females. By 96 hours after dosing the levels in kidney and liver had fallen to 0.74 µg equivs/g and 2.66 µg equivs/g respectively in males, and 1.44 and 2.57 µg equivs respectively in females. Mean concentrations in the GI tract 1 hour after dosing were 250 and 265 µg equivs/g for males and females respectively. At both dose levels the kidney, liver and GI tract were the only discrete tissues that consistently showed greater residues than in plasma. However, the GI tract residues were considered to be attributable in part to residues of GI tract contents and are therefore not relevant to tissue depletion.

Table 5.1.1-5**: Distribution of radioactivity in tissues/organs 1, 6, 12, 24, 48 and 96 hours after administration of [^{14}C]-mesotrione to rats at a dose of 1 mg/kg bw**

Tissue/Time after dosing (h)	Group mean tissue residues (µg equiv/g or mL)											
	Male						Female					
	1	6	12	24	48	96	1	6	12	24	48	96
Blood	0.366	0.020	0.007	0.003	0.001	<0.001	0.124	0.015	0.010	0.002	0.002	0.001
Plasma	0.461	0.016	0.008	0.004*	0.001	<0.001	0.088	0.014	0.010	0.002	<0.002	0.001
Brain	0.014	0.001	0.001	<0.001	<0.001	<0.001	0.006	0.001	0.001	<0.001	<0.001	<0.001
Abdom Fat	0.036	<0.010	0.002	<0.001	<0.001	<0.001	0.012	0.003	0.002	<0.001	<0.001	<0.001
Res Carcass	0.109	0.031	0.032	0.009	0.004	0.002	0.059	0.017	0.048	0.008	0.004	0.002
Heart	0.151	0.011	0.005	0.002	0.001	<0.001	0.068	0.010	0.008	0.001	0.001	0.001
Lungs	0.154	0.012	0.005	0.003	0.001	0.001	0.072	0.010	0.013	0.003	0.001	0.001
Spleen	0.087	0.009	0.003	0.001	<0.001	<0.001	0.057	0.009	0.005	0.001	<0.001	<0.001
Liver	2.923	1.848	1.645	1.599	1.425	1.297	1.644	1.835	1.595	1.425	1.236	1.009
Kidneys	3.067	0.540	0.464	0.357	0.325	0.266	1.957	0.921	0.830	0.831	0.869	0.873
G1 Tract	1.517	0.450	0.391	0.079	0.008	0.002	1.119	0.322	0.646	0.049	0.007	0.005
Thyroid	0.293*	0.080	0.257#	<0.065#	<0.679#	<0.004	0.360#	0.041	0.030#	<0.021#	<0.194#	<0.005
Thymus	0.064	0.008	0.003	0.001	<0.001	<0.001	0.036	0.008	0.006	0.001	<0.002	<0.001
Testes/ Ovaries	0.043	0.010	0.004	0.002	<0.001	<0.001	0.051	0.009	0.007	0.001	<0.002	<0.001
Pancreas	0.073*	0.009	<0.003	<0.002	<0.002	<0.002	0.035	0.007	0.005	<0.002	<0.002	<0.002
Adrenals	0.12	0.028	0.010	<0.004	<0.005	<0.003	0.034	0.020	0.012	<0.002	<0.002	<0.002
Uterus	NA	NA	NA	NA	NA	NA	0.063	0.012	0.010	0.003	0.001	0.001
Muscle	0.071	0.007	0.003	0.001	0.001	<0.001	0.025	0.006	0.005	0.001	<0.001	<0.001
Bone	0.074	0.010	0.007	<0.002	<0.001	<0.001	0.039	0.008	0.005	0.001	0.001	<0.001

n = 1 * n = 2

In terms of the proportion of administered dose present in tissues, the total amount of radioactivity in the tissues and carcass from animals dosed at 1 mg/kg bw was greater than those dosed at 100 mg/kg bw with the single exception of females 1 hour after dosing which were similar at both dose levels. Furthermore, whilst tissue concentrations were initially much higher for the 100 mg/kg dose level, by 96 hours after dosing the remaining measurable tissue residues were similar for both dose levels. There was no marked difference between the sexes, although initial concentrations were lower in females.

Table 5.1.1-6: Distribution of radioactivity in tissues/organs 1, 6, 12, 24, 48 and 96 hours after administration of [^{14}C]-mesotrione to rats at a dose of 100 mg/kg bw

Tissue/Time after dosing (h)	Group mean tissue residues (µg equiv/g or mL)											
	Male						Female					
	1	6	12	24	48	96	1	6	12	24	48	96
Blood	30.343	4.311	0.673	0.389	0.164	0.069	20.254	1.453	0.837	0.215	0.170	0.114
Plasma	41.790	5.633	0.696	0.334	0.129	<0.056	27.906	1.491	0.758	0.198	0.144	0.076
Brain	0.864	0.126	<0.133	<0.048	<0.050	<0.050	0.618	0.121	<0.105	<0.050	<0.051	<0.050
Abdom Fat	2.051	0.403	<0.119	0.098	<0.061	<0.062	2.061	0.259	<0.098	<0.062	<0.060	<0.059
Res Carcass	12.008	2.547	1.301	1.589	0.614	1.010	8.227	3.254	2.426	1.703	0.399	0.270
Heart	11.141	1.199	0.203	0.125	<0.063	<0.052	7.396	0.748	0.413	<0.071	<0.056	<0.053
Lungs	13.385	1.472	0.352	0.114	0.069	<0.054	9.243	0.832	0.474	0.134	<0.064	<0.054
Spleen	4.407	0.836	0.211	0.116	<0.044	<0.042	4.327	0.558	0.278	<0.097	<0.045	<0.042
Liver	47.463	15.163	3.025	2.553	2.347	2.656	20.997	4.169	2.952	1.964	2.387	2.565
Kidneys	175.114	20.926	4.811	1.301	0.883	0.740	115.279	13.390	4.546	1.785	1.437	1.438
G1 Tract	249.794	128.731	68.821	16.220	2.510	0.589	264.739	144.109	80.779	15.337	2.923	1.142
Thyroid	8.834	1.269	<0.592	<0.593	<0.764	<0.749	7.574	<1.057	<0.828	<2.793	<0.793	<0.976
Thymus	5.165	0.775	0.260	0.212	<0.067	<0.049	4.299	0.584	0.323	<0.075	<0.051	<0.048
Testes/ Ovaries	4.906	183.000	0.088	0.104	<0.050	<0.049	6.556	0.647	0.337	<0.112	<0.097	<0.088
Pancreas	5.986*	<1.197	0.243	0.125	<0.069	<0.070	5.033	0.833	0.348	<0.132	<0.158	<0.092
Adrenals	6.951	1.879	0.592	<0.297	<0.242	<0.256	4.447	1.581	0.598	<0.148	<0.171	<0.127
Uterus	NA	NA	NA	NA	NA	NA	12.173	3.225	0.505	0.131	0.072	<0.121
Muscle	7.846	0.976	0.229	0.126	<0.056	<0.048	3.865	0.657	0.343	0.181	<0.101	<0.049
Bone	7.413	1.148	0.509	0.489	0.214	<0.063	3.743	1.296	0.443	0.203	<0.068	<0.060

* n = 2

It was not possible to determine the terminal elimination half-lives for some tissues due to insufficient definition of the terminal phase of elimination, which, for tissues such as brain (1 mg/kg bw dose group) was a consequence of the low residue levels present and the rapidity with which radioactivity was eliminated. Approximately half of all tissue types taken were below the limit of detection by 96 hours after dosing at both dose levels. For the tissues for which half-lives were calculated these ranged from 13.8 to 250 hours following a 1 mg/kg bw dose and 15.8 to 271 hours following a 100 mg/kg dose. At 1 mg/kg, the half-lives in liver and kidney were 250 and 170 hours in males, and in liver, 147 hours in females. It was not possible to calculate a half-life for kidney in females. At 100 mg/kg bw, the half-life in kidney was 96 hours in males and 271 hours in females. It was not possible to calculate a half-life for liver for this dose group.

Table 5.1.1-7: Terminal elimination half-lives of radioactivity in tissues

Tissue	Half life (hours)			
	1mg/kg		100mg/kg	
	Males	Females	Males	Females
Blood	46.2	66.7	29.9	79.3
Plasma	46.5	70.5	29.2	52.1
Brain	*	*	*	*
Abdominal fat	82.7	*	*	*
Residual carcass	32.4	44.8	177.1	30.0
Heart	17.1	130.6	*	*
Lungs	53.0	50.0	72.0	61.2
Spleen	*	*	*	*
Liver	249.6	147.0	Not calculated	Not calculated
Kidneys	170.2	Not calculated	95.7	270.7
Gastrointestinal tract	13.8	23.6	15.8	20.6
Thyroid	*	*	*	*
Thymus	77.3	39.4	37.7	*
Ovaries	N/A	118.2	N/A	*
Testes	38.4	N/A	*	N/A
Pancreas	*	*	*	*
Adrenals	*	*	*	*
Uterus	N/A	64.8	N/A	68.7**
Muscle	42.9	82.7	58.0	39.1
Bone	101.5	59.8	24.7	46.6

N/A - not applicable; * - not calculated due to number of samples below the limit of detection; ** - 96h mean value excludes one outlier.

Discussion: Following a single oral dose of 1 mg [^{14}C]-mesotrione/kg (phase 1), the pharmacokinetic profiles in males and females were similar. Absorption of radioactivity was rapid in both sexes demonstrated by the peak concentrations occurring just 0.5 hours after dosing. The elimination of radioactivity was also fast, the initial elimination half-life being approximately 1.5 hours in males and females.

At the 100 mg/kg bw dose level, the profiles were again similar in both sexes. Absorption was slightly slower than for a 1 mg/kg dose with peak concentrations of mesotrione occurring approximately 1.5 hours after dosing in both sexes. The initial half-lives were similar to those seen following a low dose, 1.7 hours in males and 1.8 hours in females.

In the tissue depletion experiment (phase 2), all tissues showed a rapid initial rate of elimination followed by a slower terminal rate. The highest tissue concentrations were found in the kidney and liver, for both sexes at both dose levels. Residues present in the GI tract were considered to be attributable to the GI tract contents and are therefore not relevant to tissue depletion. The concentrations in all tissues declined over time with the levels in many tissues falling below the level of detection before the conclusion of the study. Although residues were still evident in kidney and liver 96 hours after dosing, the levels were seen to decline over time and the presence of residues is consistent with the role of these organs in metabolism and excretion. They may also be attributable, in part, to enterohepatic recirculation since mesotrione has been shown, in a previous study, to be eliminated via bile in both male and female rats. No other discrete tissues typically showed a greater residue than in plasma.

There were no marked sex differences in tissue concentration although initial concentrations tended to be lower in females. A comparison of tissue concentration between the 1 mg/kg bw and 100 mg/kg bw dose levels showed an initial contrast of approximately 100-fold, matching the differential in dose level. However, by 96 hours after dosing, the remaining measurable tissue residues were similar for both dose levels, thereby showing a much more extensive decline over the time course of this study.

This was consistent with many tissue concentrations falling to limit of detection values during this experiment.

It was not possible to determine the elimination half-lives for some tissues due to the insufficient definition of the terminal phase of elimination, which for tissues such as brain (1 mg/kg dose group) was a consequence of the low residue levels present and the rapidity with which radioactivity was eliminated. Residues in approximately half of all tissue types taken were below the limit of detection by 96 hours after dosing at both dose levels. However, where half-lives were calculated some were longer than the duration of the study and since the concentrations of radioactivity in some tissues, at the latter time points, were close to the limit of detection caution must be exercised when interpreting these data.

CONCLUSION:

Following a single oral dose of either 1 mg or 100 mg of mesotrione/kg bw to both male and female rats, the pharmacokinetic profiles were similar. Residues in the majority of tissues were considered to be low. The highest residues were present in the liver and kidney and were shown to decline slowly over the course of the experiment. No other discrete tissue typically showed a greater residue than in plasma. Residues in approximately half of all tissue types taken were below the limit of detection by 96 hours after dosing at both dose levels.

(Duerden A, 2005a)

Report:	KCA 5.1.1/03 Smith A, (2005). Mesotrione: Absorption, pharmacokinetics, tissue depletion and excretion study following a single oral dose (1 mg/kg or 100 mg/kg) in the mouse. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Laboratory Report No. CTL/UM0827/REGULATORY/REPORT, 28 October 2005. Unpublished. (Syngenta File No. ZA1296/2195)

Guidelines

Annex V to Council Directive 67/548/ECC, Commission Directive 87/302/EEC, OJ L133 30.5.88 (1987) (B.36. Toxicokinetics): Commission Directive 94/79/EC, Annex 1 Toxicological and Metabolism Studies No. L 354/18, 51 (1994): US EPA OPPTS 870.7485 (1998): OECD 417 (1984): JMAFF 12 Nohsan No 8147 (2000).

GLP: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

EXECUTIVE SUMMARY

The study comprised four phases during which male and female CD-1:Crl (ICR) BR mice were each given a single body weight dependent oral dose of [^{14}C]-mesotrione/kg.

Phase 1: Following a single oral dose of 1 mg/kg bw or 100 mg/kg bw the radioactivity profile and pharmacokinetic parameters in blood were determined.

Phase 2: Following a single oral dose of 1 mg/kg bw or 100 mg/kg bw the depletion of radioactivity from a range of tissues was monitored over a time course.

Phase 3: Following a single oral dose of 1 mg/kg bw to one male and one female mouse, the proportion of dose that was exhaled as volatile [^{14}C]-metabolites was determined.

Phase 4: Following a single oral dose of 1 mg/kg bw, excreta were collected at intervals for 7 days, after which the animals were terminated and a range of tissues and the residual carcass were analysed for radioactivity.

Following a single oral dose of 1 mg or 100 mg [^{14}C]-mesotrione/kg bw to male and female mice the radioactivity was very rapidly absorbed with peak blood concentrations attained approximately 1 hour after dosing in both sexes and at both doses (0.06 and 0.08 µg equivs/g in males and females after a 1 mg/kg bw dose and 5.04 and 14.3 µg equivs/g in males and females after a 100 mg/kg bw dose). Blood concentrations declined steadily and reached limit of detection values within 24 hours for the lower dose and within 6 hours for the higher dose. Apart from a higher peak concentration in female blood after the higher dose, there appeared to be no marked difference in blood profiles between the sexes.

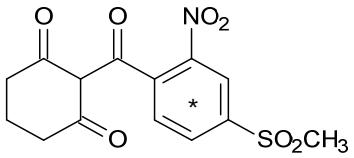
The tissue depletion results showed that following both doses, the highest tissue concentrations were attained approximately 1 hour after dosing with no major difference between the sexes. The majority of tissue concentrations declined very rapidly, reaching background values within 6 or 24 hours of dosing, irrespective of dose. In contrast, the elimination of radioactivity from liver and kidneys was slower, although the clearance from male kidneys was somewhat faster than observed in females. After 7 days, irrespective of dose, the highest residues were present in the liver with lower concentrations in the kidneys and with all other individual tissue concentrations below the limit of detection. A comparison of tissue concentrations between the 1 and 100 mg/kg bw doses showed a substantial differential for all tissues with the single exception of the liver for which the peak liver concentrations differed by only 4.5 fold in males and 2.2 fold in females. This was interpreted as showing that the capacity of the liver to retain mesotrione and its metabolites was saturable.

Following a single oral dose of 1 mg [^{14}C]-mesotrione/kg bw to male and female mice, the excretion of radioactivity was rapid. Group means of 77% to 79% of the dose were eliminated within the first 36 hours after dosing and 7% in the subsequent 132 hours. In both sexes it would appear that the greater proportion of the administered radioactivity was excreted via urine and the remainder in the faeces. When this experiment was terminated, 168 hours after dosing, the total tissue and carcass residues amounted to 7% of the dose in male mice and almost 9% in females. This was attributable almost exclusively to residues present in the liver that accounted for means of 6.8% of the dose in males and 8.2% in females. With the exception of kidneys that represented 0.05% of the dose in males and 0.5% in females, all other tissue residues were negligible. Similarly, the amount of radioactivity recovered as exhaled metabolites after a 1 mg/kg bw dose was negligible.

Following a single oral dose of 1 mg or 100 mg [^{14}C]-mesotrione/kg bw to male and female mice, the dose was rapidly absorbed and extensively distributed to tissues, with peak tissue concentrations attained at approximately 1 hour after dosing, irrespective of sex or dose level. For the vast majority of tissues, residues declined rapidly, reaching background values within 6 or 24 hours of dosing. The elimination from liver and kidneys was slower and 7 days after a 1 mg/kg bw dose, liver and kidney residues accounted for 6.8% and 0.05% of the dose respectively in males and 8.2% and 0.5% in females, whilst all other individual tissue concentrations were below the limit of detection. Tissue elimination profiles were not markedly different between the dose groups, except for liver, which appeared to have a saturable capacity to retain mesotrione residues. A 1 mg/kg bw dose was excreted predominantly in urine, showing that absorption was extensive. There was negligible radioactivity in expired air.

MATERIALS AND METHODS

Materials:

Non-Radiolabelled Test Material:	Mesotrione
Description:	Analytical grade, off white solid
Source:	Syngenta Crop Protection Inc.
Lot/Batch number:	ASW01788-01R
Purity:	99.6% (w/w)
Stability of test compound:	From information supplied by the Sponsor, the test substance was used within the stated expiry date of 31 January 2008 (stored at 4ºC).
Radiolabelled Test Material:	[^{14}C] -aromatic ring-labelled mesotrione
Specific activity:	1.426 GBq/mmol; 4.2032MBq/mg
Radiochemical purity:	>98 % [determined by TLC]
Source:	Syngenta Crop Protection Inc.
Lot/Batch number:	CL-LVII-51
Structure:	

* position of [^{14}C]-label

Vehicle: Sodium bicarbonate (10 mg/mL) in distilled water.

Preparation of dosing solutions: Non-radiolabelled (100 mg/kg dosing solution only) and [^{14}C]-radiolabelled mesotrione were recrystallised and dissolved in an aqueous solution of sodium bicarbonate (10 mg/mL) for dosing. The specific activity, homogeneity and stability of the dosing solutions were confirmed by radiochemical analysis.

Test Animals:	
Species:	Mouse
Strain:	CD-1:Crl(ICR) BR
Age/weight at dosing:	Age not reported / 20-35 g
Source:	Charles River
Housing:	In groups in stock cages or in pairs in glass metabolism cages (Group C only for the initial 24 hours after dosing)
Acclimatisation period:	5 days
Diet:	Rat and mouse No 1 diet (supplied by Special Diet Services Ltd., Stepfield, Witham, Essex, UK) *ad libitum*
Water:	Water *ad libitum*
Environmental conditions:	Temperature: 22±3°C Humidity: 30-70% Air changes: At least 15/hour Photoperiod: 12 hours light/12 hours dark

Study Design and Methods:

Experimental dates: Start: 21 March 2005 End: 06 June 2005

Group Arrangements: Animals were assigned by sex to six groups as shown below.

Group	Experiment	Dose (mg/kg bw)	Identities of mice	
			Males	Females
A	Low dose pharmacokinetics	1	1-30	31-60
B	High dose pharmacokinetics	100	61-87	88-114
C	Low dose tissue depletion	1	115-129	130-144
C*	Low dose tissue depletion	1	189-191	192-194
D	High dose tissue depletion	100	145-159	160-174
D*	High dose tissue depletion	100	183-185	186-188
E	Low dose expired air	1	129	144
F	Low dose excretion balance	1	175-178	179-182

* - Group C* and D* provided an additional time point on the terminal depletion phase.

Dosing and sample collection: A single oral bodyweight dependant dose of the appropriate dosing solution containing [^{14}C]-labelled mesotrione dissolved in aqueous sodium bicarbonate solution was administered to each mouse at a dose rate of 10 mL/kg bw.

For groups E and F, urine only was collected at 6 hours after dosing and urine and faeces were separately collected at 12 and 24 hours after dosing; additionally urine and faeces were separately collected from group F at 36 and 48 hours after dosing and then at 24 hour intervals thereafter until the termination of the study. At each collection time, each cage was rinsed with sodium bicarbonate solution and the rinsings collected together with the urine. At the end of the study, the cages were washed with sodium bicarbonate solution, which was added to the urine sample. A separate 1M aqueous hydrochloric acid wash was performed and this was retained separately.

For group E, exhaled carbon dioxide was collected for the first 24 hours after dosing.

Animals were terminated by exsanguination under terminal anaesthesia. Terminal blood samples were collected from groups A and B at 1, 2, 3, 4, 6, 8, 12, 24 and 48 hours after dosing and additionally after 96 hours (group A only). Each terminal blood sample was divided between two heparinised tubes, one of which was centrifuged to separate plasma.

Terminal tissue samples were collected from group C at 1, 3.5, 6, 24, 72 and 288 hours after dosing and from group D at 1, 2, 6, 24, 72 and 168 hours after dosing. The following tissues were taken for radioactivity measurement: adrenals, brain, heart, kidneys, liver, thyroid, uterus, lungs, testes or ovaries, pancreas, spleen, thymus and gastrointestinal tract (contents removed) together with representative samples of abdominal fat, bone (femur) and muscle; residual carcasses were retained.

All samples were analysed for radioactivity by liquid scintillation counting either directly or following tissue digestion or sample oxidation.

RESULTS AND DISCUSSION

Dose preparations: The purity, achieved concentrations and homogeneity of the dose preparations were satisfactory.

Concentrations of radioactivity in whole blood: Following a single oral dose of 1 mg [^{14}C]-mesotrione/kg bw, the pharmacokinetic profiles in males and females were similar. The peak concentrations of mesotrione (C_{max}) occurred 1 hour after dosing in both sexes (T_{max}) and were 0.06 µg equivs/g in males and 0.08 µg equivs/g in females. Blood concentrations declined steadily in both sexes and reached background values within 24 hours of dosing. There was no difference between the sexes.

Table **5.1.1-8: Concentrations of radioactivity in whole blood after administration of a single oral dose of [^{14}C]-aromatic ring labelled mesotrione to mice (1 mg/kg bw)**

Time (hours)	µg equivalents of mesotrione/g			
	Males		Females	
	Mean (n=3)	SD	Mean (n=3)	SD
1	0.059	0.021	0.080	0.008
2	0.023	0.007	0.040	0.017
3	0.048	0.051	0.048	0.042
4	0.017	0.004	0.027	0.010
6	0.006	<0.001	0.012	0.008
8	0.015	0.002	<0.005	-
12	0.008	0.004	0.012	0.007
24	<0.010	-	<0.009	-
48	<0.003	-	<0.004	-
96	<0.003	-	<0.004	-

At the 100 mg/kg bw dose level, the profiles were again similar in both sexes. The peak [^{14}C]-concentrations again occurred 1 hour after dosing in males (5.04 µg equivs/g) and in females (14.3 µg equivs/g). Despite the higher initial concentration in female blood concentrations declined rapidly in both sexes and were below the limit of detection within 6 hours of dosing for both males and females.

Table **5.1.1-9**: **Concentrations of radioactivity in whole blood after administration of a single oral dose of [^{14}C]-aromatic ring labelled mesotrione to mice (100 mg/kg bw)**

Time (hours)	µg equivalents of mesotrione/g			
	Males		Females	
	Mean (n=3)	SD	Mean (n=3)	SD
1	5.042	2.100	14.257	7.295
2	1.689	0.282	1.527	0.335
3	0.958	0.201	1.641	0.558
4	0.609	0.166	1.119	0.520
6	<0.377	-	<0.657	-
8	<0.242	-	<0.242	-
12	<0.248	-	<0.240	-
24	<0.255	-	<0.260	-
48	<0.239	-	<0.240	-

The results obtained during this phase enabled the selection of termination times for phase 2. The times selected for the 1 mg/kg bw dose level were 1, 3.5, 6, 24, 72, 168 and 288 hours after dosing and for the 100 mg/kg dose level were 1, 2, 6, 24, 72 and 168 hours after dosing.

Excretion of radioactivity: Following administration of 1 mg mesotrione/kg bw, the mean total of administered radioactivity excreted in urine and faeces over 168 hours was 84% for both male and female mice. Similarly, there was no pronounced difference in the rate of excretion between the sexes. Excretion was extensive in both sexes, with a greater proportion of the administered dose appearing to be excreted in urine by female mice compared to males. Faecal excretion accounted for mean total of 47% in male mice and 24% in female mice, while urinary excretion accounted for mean totals of 37% in male mice and 59% in female mice. However, the variability in individual animal data indicates that the faeces of some male mice were contaminated with urine; this was most marked for mouse 177M. Hence it would appear that

for both sexes the dose was excreted predominantly in urine. The residues in expired carbon dioxide were below the limit of detection at all time points. Similarly, no radioactivity was recovered in the charcoal traps. Seven days after dosing, radioactive residues in the majority of tissues were very low and the tissue distribution of radioactivity was similar for both sexes. The highest radioactive residue was found in the liver, which is consistent with the role of this organ in metabolism.

Table **5.1.1-10**: **Recovery of radioactivity in excreta after administration of a single oral dose of [^{14}C]-aromatic ring labelled mesotrione to mice (1 mg/kg bw)**

		Percentage of administered radioactive dose			
		Male		**Female**	
		Mean (n=4)	**SD**	**Mean (n=4)**	**SD**
Urine	0-6 h	4.46	-	20.25	11.46
	6-12 h	18.84	-	24.60	9.77
	12-24 h	9.43	10.40	7.92	2.50
	24-36 h	1.88	0.93	2.25	0.32
	36-48 h	0.67	0.24	1.06	0.25
	48-72 h	0.52	0.33	1.03	0.46
	72-96 h	0.40	0.17	0.64	0.17
	96-120 h	0.27	0.05	0.50	0.13
	120-144 h	0.23	0.05	0.44	0.14
	144-168 h	0.28	0.05	0.34	0.16
	Subtotal	*36.98*	*26.65*	*59.02*	*6.06*
Faeces	0-12 h	38.22	27.35	17.47	3.35
	12-24 h	4.66	0.65	3.20	1.10
	24-36 h	1.30	0.38	0.85	0.48
	36-48 h	0.94	0.33	0.75	0.36
	48-72 h	0.58	0.34	0.60	0.46
	72-96 h	0.55	0.51	0.49	0.15
	96-120 h	0.30	0.08	0.41	0.11
	120-144 h	0.23	0.03	0.21	0.10
	144-168 h	0.22	0.05	0.34	0.20
	Subtotal	*46.99*	*27.18*	*24.31*	*4.94*
Cage washes		0.18	0.02	0.34	0.15
Total excreted		*84.16*	*5.73*	*83.67*	*1.37*
Tissue, carcass, blood		6.99	0.64	8.86	1.01
Total Recovery		**91.15**	**5.14**	**92.53**	**0.46**

The total percentage recoveries of radioactivity including expired air were approximately 80% for male and 44% for female mice administered at the low dose level of 1 mg/kg. The low recovery in females is attributed to the early termination of this experiment. However, the primary purpose of this investigation was to measure the amount of radioactivity in expired air over 24 hours and this was achieved.

Tissue depletion of radioactivity: Over a 12 day time course following a single oral dose of 1 mg/kg bw [^{14}C]-mesotrione to male and female mice, both the distribution of radioactivity and its depletion from tissues were similar for both sexes. The highest tissue concentrations of radioactivity occurred 1 hour after dosing in virtually all tissues. At this time, the liver contained the highest concentration (1.6 µg equiv/g in both sexes) followed by the kidneys (0.5 and 0.8 µg equiv/g in male and female respectively) and gastrointestinal tract, although the latter tissue may have contained residues of g.i. tract contents. Progressively lower concentrations were present in other tissues with plasma concentrations of less than 0.05 µg equiv/g in both sexes. Most tissue concentrations, including plasma then declined very rapidly reaching background values by 6 or 24 hours after dosing. The only exceptions were liver, kidney, residual carcass and gastrointestinal tract, although concentrations in the latter two samples were markedly lower than hepatic or renal residues and also declined more rapidly. In males, liver concentrations declined slowly over the course of the experiment falling from 1.6 µg equiv/g after 1 hour to 0.6 µg equiv/g after 12 days. Female liver concentrations initially increased from 1.6 µg equiv/g after 1 hour to 2.1 µg equiv/g after 6 hours before falling to 1 µg equiv/g 12 days. Renal concentrations

declined faster in males falling from 0.46 µg equiv/g after 1 hour to 0.02 µg equiv/g after 12 days. Female kidney concentrations were initially higher at 0.8 µg equiv/g and declined more slowly than males with 0.5 µg equiv/g present after 12 days.

Table **5.1.1-11**: **Tissue concentrations of radioactivity after administration of a single oral dose of [^{14}C]-aromatic ring labelled mesotrione to male mice (1 mg/kg bw)**

Tissue	µg equivalents of mesotrione/g													
	1 hour		3.5 hours		6 hours		24 hours		72 hours		168 hours		288 hours	
	Mean (n=3)	SD	Mean (n=3)	SD	Mean (n=3)	SD	Mean (n=3)	SD	Mean (n=3)	SD	Mean (n=3)	SD	Mean (n=3)	SD
Blood	0.039	0.007	0.006	0.002	<0.003	-	<0.003	-	<0.003	-	<0.003	-	<0.003	-
Plasma	0.044	0.016	0.006	0.002	<0.003	-	<0.002	-	<0.001	-	<0.004	-	<0.002	-
Brain	0.014	0.001	0.003	0.001	<0.002	-	<0.001	-	<0.001	-	<0.001	-	<0.001	-
Abdominal fat	0.067	0.024	<0.033	-	<0.046	-	<0.011	-	<0.013	-	<0.006	-	<0.003	-
Residual carcass	0.076	0.019	0.032	0.006	0.031	0.011	0.006	0.002	0.004	0.001	0.002	0.001	<0.001	-
Heart	0.080	0.010	0.021	0.003	0.006	0.001	<0.003	-	<0.003	-	<0.002	-	<0.002	-
Lungs	0.089	0.010	0.018	0.002	0.007	0.002	<0.003	-	<0.002	-	<0.003	-	<0.002	-
Spleen	0.045	0.006	0.014	0.005	<0.011	-	<0.004	-	<0.003	-	<0.005	-	<0.003	-
Liver	1.593	0.177	1.541	0.151	1.529	0.132	1.219	0.039	0.761	0.087	1.022	0.120	0.621	0.144
Kidneys	0.460	0.010	0.167	0.017	0.085	0.016	0.023	0.006	0.015	0.005	0.028	0.008	0.021	0.013
G.I. tract	0.550	0.199	0.116	0.032	0.048	0.024	0.006	0.002	0.002	0.001	<0.001	-	<0.001	-
Thyroid	0.155	0.112	<0.053	-	<0.090	-	<0.031	-	<0.038	-	<0.036	-	<0.041	-
Thymus	0.051	0.001	0.100	0.147	<0.007	-	<0.007	-	<0.006	-	<0.004	-	<0.005	-
Testes	0.022	0.006	0.015	0.005	0.006	0.003	<0.002	-	<0.002	-	<0.002	-	<0.002	-
Pancreas	0.191	0.208	0.016	0.002	<0.016	-	<0.006	-	<0.004	-	<0.002	-	<0.003	-
Adrenals	0.182	0.143	<0.025	-	0.046	0.036	<0.038	-	<0.019	-	<0.008	-	<0.010	-
Muscle	0.072	0.025	0.024	0.011	0.023	0.025	<0.005	-	<0.003	-	<0.003	-	<0.002	-
Bone	0.055	0.013	0.010	0.001	<0.014	-	<0.007	-	<0.007	-	<0.018	-	<0.006	-

Table **5.1.1-12: Tissue concentrations of radioactivity after administration of a single oral dose of [^{14}C]-aromatic ring labelled mesotrione to female mice (1 mg/kg bw)**

Tissue	µg equivalents of mesotrione/g													
	1 hour		3.5 hours		6 hours		24 hours		72 hours		168 hours		288 hours	
	Mean (n=3)	SD	Mean (n=3)	SD	Mean (n=3)	SD	Mean (n=3)	SD	Mean (n=3)	SD	Mean (n=4)	SD	Mean (n=3)	SD
Blood	0.039	0.014	0.010	0.005	0.004	0.001	<0.003	-	<0.003	-	<0.004	-	<0.004	-
Plasma	0.046	0.016	0.011	0.007	0.005	0.001	<0.003	-	<0.003	-	<0.008	-	<0.004	-
Brain	0.016	0.004	0.003	0.001	<0.002	-	<0.001	-	<0.001	-	<0.001	-	<0.001	-
Abdominal fat	0.034	0.011	<0.010	-	<0.014	-	<0.006	-	<0.016	-	<0.006	-	<0.008	-
Residual carcass	0.049	0.046	0.023	0.007	0.020	0.006	0.005	0.002	0.008	0.009	0.001	0.001	<0.001	-
Heart	0.089	0.021	0.017	0.004	0.010	0.002	<0.003	-	<0.004	-	<0.002	-	<0.003	-
Lungs	0.083	0.023	0.016	0.003	0.015	0.007	<0.009	-	<0.003	-	<0.002	-	<0.002	-
Spleen	0.047	0.006	0.007	0.002	<0.005	-	<0.004	-	<0.004	-	<0.005	-	<0.003	-
Liver	1.611	0.501	1.838	0.211	2.099	0.201	2.077	0.255	1.762	0.397	1.418	0.197	0.971	0.179
Kidneys	0.795	0.137	0.427	0.045	0.415	0.162	0.353	0.073	0.428	0.041	0.346	0.132	0.471	0.145
G.I. tract	0.468	0.296	0.170	0.024	0.149	0.012	0.010	0.003	0.003	0.001	<0.001	-	<0.001	-
Thyroid	0.236	0.195	<0.103	-	<0.036	-	<0.035	-	<0.236	-	<0.048	-	<0.016	-
Thymus	0.054	0.013	<0.008	-	<0.009	-	<0.004	-	<0.005	-	<0.006	-	<0.004	-
Ovaries	0.052	0.011	0.015	0.005	0.010	0.007	<0.005	-	<0.009	-	<0.005	-	<0.005	-
Pancreas	0.145	0.096	0.014	0.004	0.009	0.001	<0.005	-	<0.004	-	<0.003	-	<0.003	-
Adrenals	0.087	0.026	0.025	0.004	<0.061	-	<0.012	-	<0.012	-	<0.015	-	<0.010	-
Uterus	0.050	0.015	0.014	0.005	0.010	0.006	<0.005	-	<0.003	-	<0.004	-	<0.003	-
Muscle	0.055	0.009	0.019	0.001	<0.013	-	<0.005	-	<0.003	-	<0.002	-	<0.003	-
Bone	0.036	0.003	<0.019	-	<0.010	-	<0.010	-	<0.008	-	<0.016	-	<0.007	-

Over a 7 day time course following a single oral dose of 100 mg/kg bw [^{14}C]-mesotrione to male and female mice, both the distribution of radioactivity and its depletion from tissues were similar for both sexes, as seen at the low dose level. The highest tissue concentrations of radioactivity also occurred 1 hour after dosing in virtually all tissues. At this time the gastrointestinal tract contained the highest concentration (40 and 60 µg equivs/g in males and females respectively) although this may be in part attributable to residues of g.i. tract contents. The highest individual tissue concentration in both sexes was present in the kidneys (37 µg equivs/g in male and 32 µg equivs/g in female). In contrast to the low dose level, hepatic concentrations (7 and 4 µg equivs/g in males and females respectively) were substantially lower than renal values. At 1 hour after dosing the radioactivity in the residual carcass accounted for almost 28 µg equivs/g in males and just 7 µg equivs/g in females. All other tissue concentrations were similar to or lower than that present in plasma (5.5 and 7 µg equivs/g in males and females respectively). Most tissue concentrations, including plasma then declined very rapidly, reaching background values by 6 or 24 hours after dosing. The only exceptions were liver, kidney, male blood, residual carcass and gastrointestinal tract; although concentrations in the latter sample declined as the test material was excreted. The measurable residues in male blood until 72 hours after dosing are not consistent with the pharmacokinetics results at this dose level, which showed that male blood concentrations declined to background values by 6 hours after dosing. There was no other evidence of any binding to blood cells, hence, the depletion of plasma concentrations to limit of detection values by 6 hours after dosing is taken to reflect effective clearance. In male mice, the liver concentration declined slowly over the course of the experiment falling from 7 µg equiv/g after 1 hour to 2.5 µg equivs/g after 7 days. In female mice the hepatic concentration initially increased from 4 µg equivs/g after 1 hour to 4.6 µg equiv/g after 2 hours before falling to 3 µg equivs/g by 7 days. Renal concentrations declined steadily in males falling from 37 µg equivs/g after 1 hour, to 0.5 µg equiv/g after 24 hours and to below the limit of detection after 7 days. Female kidney concentrations declined slightly more slowly from 32 µg equivs/g after 1 hour, to 1.2 µg equiv/g after 24 hours and to 0.6 µg equiv/g after 7 days. By the end of this experiment, only the liver and residual carcass concentrations were above the limit of detection in male mice, with these same tissues and the kidney as the only tissue concentrations above background values in females.

A direct comparison of tissue concentrations between the dose levels is inappropriate because of differences in the tissue sampling times. However, whilst most tissue concentrations show a substantial difference between the dose levels, the liver did not with differentials in peak concentrations of only 4.5 and 2.2 fold in males and females respectively. This suggests that the capacity of the liver to retain mesotrione or its metabolites is saturable.

Table **5.1.1-13: Tissue concentrations of radioactivity after administration of a single oral dose of [^{14}C]-aromatic ring labelled mesotrione to male mice (100 mg/kg bw)**

Tissue	µg equivalents of mesotrione/g											
	1 hour		2 hours		6 hours		24 hours		72 hours		168 hours	
	Mean (n=3)	SD	Mean (n=3)	SD	Mean (n=3)	SD	Mean (n=3)	SD	Mean (n=3)	SD	Mean (n=3)	SD
Blood	3.999	1.949	4.594	2.772	1.605	0.955	1.666	0.095	0.405	0.093	<0.133	-
Plasma	5.505	2.481	2.365	1.499	<0.438	-	<0.296	-	<0.287	-	<0.290	-
Brain	0.857	0.703	0.302	0.071	<0.163	-	<0.086	-	<0.097	-	<0.087	-
Abdominal fat	5.890	3.719	<1.051	-	<1.299	-	<0.959	-	<0.887	-	<0.843	-
Residual carcass	27.771	15.648	5.514	1.620	9.676	4.832	1.273	1.001	0.368	0.056	0.518	0.540
Heart	2.612	0.869	1.519	0.335	1.213	1.142	<0.233	-	<0.224	-	<0.184	-
Lungs	3.210	1.134	1.519	0.305	1.067	0.128	<0.170	-	<0.173	-	<0.157	-
Spleen	3.585	4.293	1.406	0.955	0.496	0.102	<0.313	-	<0.317	-	<0.381	-
Liver	7.112	1.967	4.797	1.524	2.736	0.907	3.138	1.763	2.216	0.160	2.510	0.048
Kidneys	37.370	10.641	5.937	1.780	5.653	2.704	0.473	0.059	0.157	0.037	<0.116	-
G.I. tract	39.976	35.269	27.057	9.449	20.048	7.068	0.661	0.131	<0.225	-	<0.151	-
Thyroid	12.201	14.722	<4.277	-	<5.024	-	<2.245	-	<3.916	-	<1.796	-
Thymus	3.274	1.618	2.002	1.027	1.356	1.064	<0.418	-	<0.509	-	<0.573	-
Testes	6.463	4.207	1.894	1.706	1.756	1.367	<0.218	-	<0.200	-	<0.181	-
Pancreas	5.127	5.665	2.082	1.088	0.724	0.218	<0.261	-	<0.270	-	<0.213	-
Adrenals	2.440	1.234	<1.020	-	0.786	0.393	<0.722	-	<0.728	-	<0.699	-
Muscle	3.898	2.126	1.708	1.000	2.598	2.892	<1.034	-	<0.234	-	<0.249	-
Bone	<4.869	-	<1.597	-	<2.183	-	<1.017	-	<1.124	-	<0.497	-

Table **5.1.1-14: Tissue concentrations of radioactivity after administration of a single oral dose of [^{14}C]-aromatic ring labelled mesotrione to female mice (100 mg/kg bw)**

Tissue	µg equivalents of mesotrione/g											
	1 hour		2 hours		6 hours		24 hours		72 hours		168 hours	
	Mean (n=3)	SD	Mean (n=3)	SD	Mean (n=3)	SD	Mean (n=3)	SD	Mean (n=3)	SD	Mean (n=3)	SD
Blood	0.757	0.615	<0.170	-	<0.262	-	<0.260	-	<0.143	-	<0.162	-
Plasma	7.249	2.631	1.914	0.123	0.614	0.147	<0.296	-	<0.293	-	<0.289	-
Brain	0.351	0.079	0.223	0.081	0.203	0.071	<0.086	-	<0.088	-	<0.089	-
Abdominal fat	1.586	0.502	0.941	0.331	<0.396	-	<0.380	-	<0.635	-	<1.404	-
Carcass	6.830	1.202	2.408	1.425	4.937	0.902	0.392	0.146	0.195	0.039	0.288	0.071
Heart	2.501	0.744	1.440	0.525	0.545	0.151	<0.216	-	<0.245	-	<0.222	-
Lungs	3.226	1.087	1.332	0.391	0.517	0.121	<0.228	-	<0.170	-	<0.166	-
Spleen	1.753	0.444	1.654	0.563	<0.379	-	<0.307	-	<0.311	-	<0.356	-
Liver	3.980	1.216	4.633	1.882	2.747	1.030	2.546	0.546	3.495	0.327	2.906	0.463
Kidneys	31.652	10.395	11.469	5.027	3.911	1.201	1.161	0.609	0.782	0.029	0.634	0.124
G.I. tract	59.159	14.033	39.605	7.088	14.475	4.718	1.071	0.625	0.215	0.016	<0.133	-
Thyroid	<6.391	-	<4.791	-	<2.114	-	<1.649	-	<2.033	-	<1.688	-
Thymus	1.562	0.311	1.217	0.437	0.683	0.161	<0.305	-	<0.345	-	<0.315	-
Testes	2.418	0.545	1.748	0.643	<0.798	-	<0.480	-	<0.400	-	<0.505	-
Pancreas	5.387	5.859	1.894	1.239	0.684	0.114	<0.294	-	<0.274	-	<0.251	-
Adrenals	2.847	0.681	1.371	0.389	<0.502	-	<0.483	-	<0.872	-	<0.668	-
Uterus	5.548	0.666	2.259	1.561	0.619	0.168	<0.506	-	<0.360	-	<0.364	-
Muscle	2.805	1.930	1.453	0.549	<0.776	-	<0.264	-	<0.300	-	<0.295	-
Bone	1.778	0.872	1.398	0.400	<0.987	-	<1.150	-	<1.437	-	<0.501	-

Total recovery of radioactivity: At the low dose level of 1 mg/kg, the total mean percentage recoveries of administered radioactivity including excreta, tissues and residual carcasses were approximately 91% for males and 93% for females.

Tissue half lives of radioactivity:

1 mg/kg dose: No terminal half lives of elimination values are reported for the majority of tissues because residues declined so rapidly that background values were attained within 24 hours of dosing. The terminal half lives for residues in the residual carcass were 4.3 days for male mice and 3.7 days for females, with an estimate of 10.5 days for female liver. The observed inter-animal variability for male liver, kidneys and g.i. tract precluded the calculation of reliable half life estimates for these tissues.

100 mg/kg dose: As for the 1mg/kg dose group, no terminal half life values were calculated for the majority of tissues because residues declined so rapidly that background values were attained within 24 hours of dosing. The terminal half life for kidney residues was 3.3 days for male mice and 7.4 days for females, with estimates of 3.1 and 2.2 days for the g.i. tract in male and female mice. The observed inter-animal variability for liver and residual carcass residues made the calculation of half life estimates unreliable.

Table **5.1.1-15: Half lives of radioactivity in tissues after administration of a single oral dose of [^{14}C]-aromatic ring labelled mesotrione**

Tissue	Half live (days)			
	1 mg [^{14}C]-mesotrione/kg bw		100 mg [^{14}C]-mesotrione/kg bw	
	Male	Female	Male	Female
Blood	NA*	NA*	41.5	NA*
Plasma	NA*	NA*	NA*	NA*
Brain	NA*	NA*	NA*	NA*
Abdominal fat	NA*	NA*	NA*	NA*
Residual carcass	4.3	3.7	NC*	NC*
Heart	NA*	NA*	NA*	NA*
Lungs	NA*	NA*	NA*	NA*
Spleen	NA*	NA*	NA*	NA*
Liver	NC*	10.5	NC*	NC*
Kidneys	NC*	NC*	3.3	7.4
G.I. tract	NC*	NC*	3.1	2.2
Thyroid	NA*	NA*	NA*	NA*
Thymus	NA*	NA*	NA*	NA*
Ovaries	NA*	NA*	NA*	NA*
Testes	NA*	NA*	NA*	NA*
Pancreas	NA*	NA*	NA*	NA*
Adrenals	NA*	NA*	NA*	NA*
Uterus	NA*	NA*	NA*	NA*
Muscle	NA*	NA*	NA*	NA*
Bone	NA*	NA*	NA*	NA*

NA* – Terminal half life values not calculated on these tissues samples due to the data values being equal to or below the limit of detection by 24 hours post dose. NC* – Terminal half life values could not be calculated with confidence because of inter-animal variability.

Discussion; Following a single oral dose of 1 mg or 100 mg [^{14}C]-mesotrione/kg bw to male and female mice the radioactivity was very rapidly and extensively absorbed with peak blood concentrations attained approximately 1 hour after dosing in both sexes and at both dose levels. Blood concentrations declined steadily and reached limit of detection values within 24 hours at the low dose level and within 6 hours at the high dose level. Apart from a higher peak concentration in female blood at the high dose level, there appeared to be no marked difference in blood profiles between the sexes.

Following a single oral dose of 1 mg [^{14}C]-mesotrione/kg bw to male and female mice, the excretion of radioactivity was rapid. Group means of 77% to 79% of the dose were eliminated within the first 36 hours after dosing and 7% in the subsequent 132 hours. In both sexes it would appear that the greater proportion of the administered radioactivity was excreted via urine and the remainder in the faeces. Female mice excreted a mean of 59% of the dose in urine and 24% in faeces. The corresponding results for males showed a group mean of just 37% in urine and 47% in faeces; however, the marked inter-animal variability shown suggests that for male 177, most of the urinary radioactivity excreted within the first 12 hours of dosing was absorbed by faecal pellets adherent to the walls of the metabolism cage. Furthermore, some urine could have been similarly absorbed onto faecal material for other mice, hence, it is concluded that the reported urinary excretion data are likely to underestimate the total urinary excretion. It therefore follows that absorption of mesotrione by the mouse is greater than the urinary and tissue measurements estimate. At the termination of the excretion study, the gastrointestinal tract contained less than 0.03% of the dose for each sex, confirming that faecal elimination was essentially complete. When this experiment was terminated, 168 hours after dosing, the total tissue and carcass residues amounted to 7% of the dose in male mice and almost 9% in females. This was attributable almost exclusively to residues present in the liver that accounted for means of 6.8% of the dose in male mice and 8.2% in females. With the exception of kidneys that represented 0.05% of the dose in males and 0.5% in females, all other tissue residues were negligible. Similarly, the amount of radioactivity recovered as exhaled volatile metabolites after a 1 mg/kg bw dose level was negligible.

The tissue depletion experiments showed that for both dose groups the highest tissue concentrations were attained approximately 1 hour after dosing and with no major differences between the sexes. The majority of tissue concentrations declined very rapidly, reaching background values within 6 or 24 hours of dosing, irrespective of dose. Hence, for these tissues, no terminal half life of elimination could be calculated. The elimination of radioactivity from liver and kidneys was slower, although the clearance from male kidneys was somewhat faster than observed in females. After 7 days, irrespective of dose, the highest tissue residues were present in the liver with lower concentrations in the kidneys and with all other individual tissue concentrations below the limit of detection. A comparison of tissue concentrations between the 1 mg/kg bw and 100 mg/kg bw dose groups showed a substantial differential for all tissues with the single exception of the liver for which the peak liver concentrations differed by only 4.5 fold in males and only 2.2 fold in females. This was interpreted as showing that the capacity of the liver to retain mesotrione and its metabolites was saturable. In addition to the liver and kidneys, the rate of depletion of radioactivity was also relatively slow from the gastrointestinal tract and the residual carcass. However, the g.i. tract concentrations may have included residues of gut contents and these measurements were associated with the established faecal route of excretion of radioactivity. The residual carcass measurements would be attributable in part to the redistribution of radioactivity as other tissue residues, including the liver and kidneys, declined. The inter-animal variability in measurements made the calculation of terminal half lives of elimination difficult to estimate for liver, kidneys, g.i. tract and residual carcass. However, for those tissues showing a steady decline in residues, the terminal half lives ranged from 2.2 to 10.5 days. These estimates are therefore considered to reasonably reflect terminal elimination characteristics.

CONCLUSION:

Following a single oral dose of 1 mg or 100 mg [^{14}C]-mesotrione/kg bw to male and female mice, the dose was rapidly absorbed and extensively distributed to tissues, with peak tissue concentrations attained at approximately 1 hour after dosing, irrespective of sex or dose. For the vast majority of tissues, residues declined rapidly, reaching background values within 6 or 24 hours of dosing. The elimination from liver and kidneys was slower and 7 days after a 1 mg/kg bw dose, liver and kidney residues accounted for 6.8% and 0.05% of the dose respectively in males and 8.2% and 0.5% in females, whilst all other individual tissue concentrations were below the limit of detection. Tissue elimination profiles were not markedly different between the doses, except for liver which appeared to have a saturable capacity to retain mesotrione residues. A 1 mg/kg bw dose was excreted predominantly in urine, showing that absorption was extensive. There was negligible radioactivity in expired air.

(Smith AD, 2005)

CA 5.1.2 Absorption, distribution, metabolism and excretion by other routes

Please refer to original EU review. No new data or assessment is provided.

Relevant literature on absorption, distribution, metabolism and excretion of mesotrione.

Scientifically peer-reviewed open literature was identified as follows:

Reference	Categorisation and Comments	Summary
Hall MG, Wilks MF, McLean Provan W, Eksborg S and Lumholtz B (2001) Pharmacokinetics and pharmacodynamics of NTBC (2-(2-nitro-4-fluoromethylbenzoyl)-1,3-cyclohexanedione) and mesotrione, inhibitors of 4-hydroxyphenyl pyruvate dioxygenase (HPPD) following a single dose to healthy male volunteers. Br J Clin Pharmacol **52**, 169-177	For mesotrione paper summarises data reported in Hall (1997) reviewed in **Mesotrione (ZA1296) DAR December 1999**. It compares this with data derived from human volunteer studies with the HPPD inhibiting drug nitisinone (Orfadin®) also known as NTBC. Data are derived from human volunteer studies performed in accordance with the declaration of Helsinki (1964) and are considered reliable.	NTBC and mesotrione are both inhibitors of HPPD, although the magnitude and duration of their effect on tyrosine concentrations are very different. When normalized for dose, the extent of the induced tyrosinaemia after administration of NTBC and over the duration of these studies was approximately 400 fold greater than that following administration of mesotrione. The persistent and significant effect on HPPD following administration of NTBC makes it suitable for the treatment of patients with hereditary tyrosinaemia type 1 (HT-1), whilst the minimal and transient effects of mesotrione minimize the likelihood of a clinical effect in the event of systemic exposure occurring during occupational use.
Gledhill AJ, Jones BK and Laird WJD (2001): Metabolism of 2-(4-methylsulphonyl-2-nitrobenzoyl)-1,3-cyclohexanedione (mesotrione) in rat and mouse. Xenobiotica **31(10)** 733-747	The paper summarises data on ADME studies in the mouse and rat previously evaluated in **Mesotrione (ZA1296) DAR, Volume 3.** Data are from OECD guideline studies and are considered reliable	The paper does not present any new data but considers the association of the ADME data from both species in the context of the species difference in toxicity. Mesotrione was extensively absorbed and rapidly excreted via urine in both rat and mouse. The absorbed dose was not well metabolized in either species. Unabsorbed material was subject to metabolic action by the gut microflora. The major metabolic pathway was hydroxylation of the aromatic ring. There was evidence for cleavage of the dione and aromatic rings followed by reduction of the nitro group in the gastrointestinal tract. There were no species differences in the metabolism and excretion of mesotrione, which could explain the species differences in toxicity reported for this class of compounds.

A full reliability assessment as described in MCA Section 9 has not been undertaken for these references as data on mesotrione have been evaluated as part of **Mesotrione (ZA1296) DAR, Volume 3**

CA 5.2 Acute Toxicity

CA 5.2.1 Oral

Please refer to original EU review. No new data or assessment is provided.

CA 5.2.2 Dermal

Please refer to original EU review. No new data or assessment is provided.

CA 5.2.3 Inhalation

Please refer to original EU review. No new data or assessment is provided.

CA 5.2.4 Skin irritation

Please refer to original EU review. No new data or assessment is provided.

CA 5.2.5 Eye irritation

Please refer to original EU review. No new data or assessment is provided.

CA 5.2.6 Skin sensitisation

Please refer to original EU review. No new data or assessment is provided.

CA 5.2.7 Phototoxicity

Table 5.2.7-1: EU Conclusions – Phototoxicity

Property	EU agreed endpoint (Mesotrione: SANCO/1416/2001 – Final)	Proposed endpoint
Phototoxicity	-	Not phototoxic

A phototoxicity study is included in response to the new data requirements.

Report:	KCA 5.2.7/01 Lehmeier D (2013). Mesotrione Technical: *In Vitro* 3T3 NRU Phototoxicity Test. BSL Bioservice, Scientific Laboratories GmbH, Behringstrasse 6/8, 82152 Planegg, Germany. Laboratory Report No. 132889, 27 September 2013. Unpublished. Syngenta File No. ZA1296_10183

Guideline: Phototoxicity – *in vitro* 3T3 NRU test; OECD 432 (2004): OECD 101 (1981): 440/2008/EC (2008).

GLP: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

EXECUTIVE SUMMARY

The phototoxic potential of mesotrione technical (86.1% mesotrione wet paste) was determined in an *in vitro* cytotoxicity assay with the BALB/3T3 mouse fibroblast cell line. The basis of this test is a comparison of the cytotoxicity of the test item when tested in the presence and in the absence of exposure to a non-cytotoxic dose of UVA light and cytotoxicity is expressed as a concentration dependent reduction of the uptake of the vital dye neutral red.

Mesotrione technical was dissolved in DMSO and diluted in a 1:100 ratio in Earle‘s balanced salt solution. BALB/3T3 cells were treated for 1 hour with different concentrations of the test solution at 37±1°C and for a further 50 min in the absence and presence of a non-toxic dose of UVA light. One day after treatment cytotoxicity was analysed as a measure of reduction of neutral red uptake and compared to the controls.

In this study under the given conditions the test item showed no cytotoxic effects, neither with nor without irradiation. The EC_{50}-values could not be determined and therefore a PIF could not be calculated.

Mesotrione technical is classified as “non phototoxic”.

MATERIALS AND METHODS

Materials:

Test Material:	Mesotrione Technical
Description:	Technical, brown solid
Lot/Batch number:	631795
Supplied by:	Syngenta Crop Protection Monthey AG, Switzerland
Purity:	86.1% w/w mesotrione wet paste (*determined by GC, HPLC, LC/MS, IC, Mass spectrometry, Karl Fischer*)
Contaminants:	Not reported
CAS#:	104206-82-8
Stability of test compound:	Until end February 2016 (stored at <30°C)

Media:
Cell culture medium: Dulbecco’s Modified Eagle Medium (DMEM, 10x; Biochrom) with 4.5 g/L D-glucose. The medium was diluted 1:10 with A. dest. (Sigma) and supplemented with the following items:
10% New Born Calf Serum (NCS)
1% Penicillin/Streptomycin (final concentration: 100 IU/100 µg/mL)
1% Amphotericin B (final concentration: 2.5 µg/mL)
2% L-glutamine (final concentration: 4 mM)
2% NaHCO3 (final concentration: 1.5 g/L)
1% Na-pyruvate (final concentration: 1 mM)
Neutral Red (NR) Stock Solution
0.4 g neutral red
100 mL H_2O
NR Medium
1 mL NR stock solution
79 mL cell culture medium (without NCS)

Controls: Negative (untreated): 1% Dimethylsulfoxide (DMSO) in Earle‘s balanced salt solution (EBSS). Positive: Chlorpromazine (2-Chloro-10-[3-dimethylaminopropyl] phenothiazine.

Study Design and Methods:

Experimental dates: Start: 03 June 2013 End: 06 June 2013

Preparation of test item: Mesotrione technical was dissolved in DMSO and diluted in EBSS (Earle's balanced salt solution) in a 1:100 ratio to a highest final concentration of 1000 µg/mL (stock solution). From the stock solution, concentrations of the test item were prepared. In all mesotrione concentrations, the solvent was present at a constant volume ratio of 1% (v/v).

Cells: The test was carried out with BALB/3T3 cells. Cells from frozen stock cultures, tested routinely for mycoplasma, were seeded in culture medium at an appropriate density and subcultured at least once before they were used in the *in vitro* 3T3 NRU phototoxicity test. Cells at passage number 83 were used. Cells were precultured in 75 cm^2 culture flasks in DMEM with 10% new born calf serum at 37 ± 1°C and 5% CO_2.

The UVA-sensitivity of the cells was determined and found to be acceptable. Six microtiter plates with cells were irradiated with the UVA-doses 0 (dark control), 3, 5, 7, 9 and 11 J/cm^2. The cells meet acceptance criteria if their viability after irradiation with 5 J/cm^2 is not less than 80% and after irradiation with 9 J/cm^2 is not less than 50%

Doses: 1000; 316; 100; 31.6, 10.0; 3.16; 1.00 and 0.316 µg mesotrione technical/mL, plus negative control (1% DMSO in EBSS), blank (EBSS) and positive control (Chlorpromazine 100; 31.6, 10.0; 3.16; 1.00; 0.316; 0.100 and 0.0316 µg/mL without UVA and 10; 3.16; 1.00; 0.316; 0.100, 0.0316, 0.0100, and 0.00316 µg/mL with UVA).

Experimental procedure: A cell suspension of 1×10^5 cells/mL in culture medium was prepared. 100 µL culture medium were dispensed into the peripheral wells of a 96-well tissue culture microtiter plate (blanks). In the remaining wells, 100 µL of a cell suspension of 1×10^5 cells/mL (1×10^4 cells/well) were dispensed. For each test item two plates were prepared: one for determination of cytotoxicity (without UVA), and the other for determination of photocytotoxicity (with UVA).

The cells were incubated for 24±2 h (5% CO_2, 37±1°C) until they formed a half-confluent monolayer. This incubation period allowed for cell recovery and adherence, and for exponential growth.

After incubation, cells were washed with 150 µL EBSS per well.

The solutions of the test item and the positive control were diluted seven times at a ratio of √10. The positive control was tested in a full scale phototoxicity test on two plates in parallel to the test item. 8 different concentrations were applied to 6 parallel cultures each.

100 µL of the appropriate concentration of test item or solvent only (negative control) were added to the cells. The cells were then incubated in the dark for 60 minutes (5% CO_2, 37±1°C).

The cells were irradiated for 50 min through the lid of the 96-well plate with 1.5-1.7 mW/cm2 UVA (= 4.5-5.1 J/cm^2). The positions of the plates were exchanged after half time of the irradiation (25 min.). Duplicate plates (without UVA) were kept at room temperature in a dark box for 50 min.

After exposition the cells were washed with 150 µL EBSS. EBSS was replaced with culture medium and the plates were incubated (5% CO_2, 37±1°C) overnight (18-22 hours). Following the incubation the cells were washed with 150 µL EBSS. 100 µL neutral red (NR) medium were added and the plates were incubated at 37 ± 1°C, in a humidified atmosphere of 5% CO_2, for 3 hours.

After incubation, the NR medium was removed and the cells were washed with 150 µL EBSS. 150 µL NR desorb solution (freshly prepared ethanol/acetic acid) was added. The microtiter plate was shaken

rapidly on a microtiter plate shaker for 10 min, until the NR had been extracted from the cells and had formed a homogeneous solution. Then the optical density of the NR extract was measured at 550 nm in a micro plate auto reader, using blanks as a reference.

Data analysis: Relative cell viability, expressed as percentage of untreated controls, was calculated for each of the eight test concentrations. To predict the phototoxic potential, the concentration responses obtained in the presence (+UVA) and in the absence (-UVA) of irradiation were compared at the EC_{50} level, i.e. at the concentration inhibiting cell viability by 50% in comparison with untreated controls.

If complete concentration response curves are obtained, both in the presence (+UVA) and in the absence (-UVA) of light, a photo-irritation-factor (PIF) is calculated by means of the following formula:

$$PIF = \frac{EC_{50}\ (-UVA)}{EC_{50}\ (+UVA)}$$

Interpretation - PIF < 2: "no phototoxicity", PIF > 2 and < 5: "probable phototoxicity", PIF > 5: "phototoxicity". If both EC_{50} (-UVA) and EC_{50} (+UVA) cannot be calculated due to the fact that a test item does not show any cytotoxicity up to the highest concentration, this indicates no phototoxic potential.

If a test item is only cytotoxic +UVA and is not cytotoxic when tested -UVA, the PIF cannot be calculated, although this result indicates a phototoxic potential of the test item. In such cases the mean photo effect (MPE) is analysed. The MPE is based on comparison of the complete concentration response curves and is defined as the weighted average across a representative set of photo effect values. MPE < 0.1: "no phototoxicity", - MPE > 0.1 and < 0.15: "probable phototoxicity", - MPE > 0.15: "phototoxicity".

Statistics: Data was analysed using the Phototox Version 2.0 Software (***Peters B. and Holzhütter HG, 2002 and Bundesinstitut für Risikobewertung {Federal Institute for Risk Assessment, BfR***})

RESULTS AND DISCUSSION

The cells treated with mesotrione technical showed no cytotoxic effects, either with or without irradiation. At the highest mesotrione concentration (1000 µg/mL), viability of the non-irradiated cells was 102.4% relative to the untreated negative controls. After irradiation, cell viability was reduced to 87.3% and, therefore, no EC_{50} values and no PIF could be determined. This indicates no phototoxic potential.

The controls confirmed the validity of the study. The negative controls of the +UVA experiment showed a viability of 93.9% of the untreated controls. The mean OD_{550} of the untreated controls was ≥0.4. The cells treated with the positive control showed cytotoxic and phototoxic effects. At the highest positive control concentration without irradiation (100 µg/mL), viability of the cells was reduced to 0% relative to the untreated negative controls. The EC_{50} value was calculated to be 13.96 µg/mL. At the highest positive control concentration with irradiation (10 µg/mL), the EC_{50} value was calculated to 0.55 µg/mL and the PIF was >6 (25.4).

Table 5.2.7-1: **Mean OD_{550} and cell viability (%) with and without UVA irradiation**

Compound	Without UVA			With UVA		
	Concentration (µg/mL)	Mean value OD_{550nm}	% viability	Concentration (µg/mL)	Mean value OD_{550nm}	% viability
Mesotrione	1000.00	0.512	102.4	1000.00	0.393	87.3
	316.23	0.492	98.3	316.23	0.425	94.4
	100.00	0.506	101.1	100.00	0.437	97.0
	31.62	0.489	97.8	31.62	0.458	101.7
	10.00	0.503	100.5	10.00	0.449	99.7
	3.16	0.504	100.7	3.16	0.451	100.1
	1.00	0.500	99.8	1.00	0.439	97.4
	0.316	0.493	98.6	0.316	0.443	98.3
Chlorpromazine	100.00	0.000	0.0	10.00	0.003	0.6
	31.62	0.000	0.0	3.16	0.000	0.0
	10.00	0.352	74.3	1.00	0.006	1.2
	3.16	0.467	98.5	0.316	0.413	89.0
	1.00	0.476	100.5	0.100	0.454	97.8
	0.316	0.480	101.4	0.0316	0.467	100.7
	0.100	0.471	99.5	0.0100	0.477	102.7
	0.0316	0.464	98.1	0.00316	0.476	102.6

CONCLUSION: Mesotrione technical is considered to be “non phototoxic”.

REFERENCES:

Peters B. and Holzhütter HG (2002), *In Vitro* Phototoxicity Testing: Development and Validation of a New Concentration Response Analysis Software and Biostatistical Analyses Related to the Use of Various Prediction Models, *ATLA* **30**, pp. 415-432.

Bundesinstitut für Risikobewertung (Federal Institute for Risk Assessment, BfR), Zentralstelle zur Erfassung und Bewertung von Ersatz- und Ergänzungsmethoden zum Tierversuch (Centre for the Documentation and Evaluation of Alternatives to Animal Experiments, ZEBET), Phototox Version 2.0, “How to get started“.

(Lehmeier D, 2013)

CA 5.3 Short-Term Toxicity

CA 5.3.1 Oral 28-day study

Please refer to original EU review. No new data or assessment is provided.

CA 5.3.2 Oral 90-day study

Please refer to original EU review. No new data or assessment is provided.

CA 5.3.3 Other routes

Please refer to original EU review. No new data or assessment is provided.

CA 5.4 Genotoxicity Testing

CA 5.4.1 *In vitro* studies

A new guideline bacterial reverse mutation assay has been conducted to reflect the new specification for mesotrione technical material. Details can be found in **Document J**.

Report:	KCA 5.4.1/01 Sokolowski A (2013). Mesotrione –Salmonella Typhimurium and Escherichia Coli Reverse Mutation Assay. Harlan Cytotest Cell Research GmBH, Rossdorf, Germany . Report No. 1537300, 04 July 2013. Unpublished. Syngenta File No. ZA1296_10127

Guideline: Reverse Mutation Test Using Bacteria. OECD 471 (1997): OPPTS 870.5100 (1998): EC440/2008 B.13/B.14 (2008)

GLP: Signed and dated GLP and Quality Assurance statements were provided. There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study

EXECUTIVE SUMMARY

In a reverse gene mutation assay in bacteria, strains of *S. typhimurium* (TA1535, TA1537, TA98 and TA100) and strains of *E. coli* (WP2*uvrA* pKM101 and WP2 pKM101) were exposed to mesotrione (purity 96.6% w/w) at concentrations of 3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate in the presence and absence of mammalian metabolic activation in the plate incorporation test (experiment I) and at 33, 100, 333, 1000, 2500 and 5000 µg/plate in the presence and absence of mammalian metabolic activation in the pre-incubation test (experiment II).

The plates incubated with mesotrione showed normal background growth up to the limit concentration of 5000 µg/plate in the presence and absence of mammalian metabolic activation in both independent experiments.

No cytotoxic effects, evident as a reduction in the number of revertants occurred in the test groups in the presence and absence of mammalian metabolic activation in both independent experiments.

No increase in revertant colony numbers of any of the six tester strains was observed following treatment with mesotrione at any concentration level, in the presence or absence of mammalian metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance. Appropriate reference mutagens were used as positive controls and showed a distinct increase of induced revertant colonies.

There was no evidence of induced mutant colonies over background. Mesotrione did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used. Mesotrione is considered to be non-mutagenic in the *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay.

MATERIALS AND METHODS

Materials:

Test Material:	Mesotrione
Description:	Technical, beige powder
Lot/Batch number:	587943 (SM07F377)
Purity:	96.6% w/w a.i
CAS#:	Not reported
Stability of test compound:	Stability confirmed until May 2013 (stored at room temperature)

Control Materials:

Negative:	Yes
Solvent control (final concentration):	DMSO 10µL/plate
Positive control:	Nonactivation: Sodium azide, NaN_3: 10 µg/plate TA100, TA1535 4-Nitro-o-phenylene-diamine, 4-NOPD: 10 µg/plate TA98, 50 µg/plate TA1537 Methyl methane sulfonate, MMS: 2.0 µg/plate WP2 *uvrA* (pKM101) and WP2 (pKM101) Activation: 2-Aminoanthracene, 2-AA 2.5 µg/plate TA1535, TA1537, TA98, TA100 10 µg/plate WP2 *uvrA* (pKM101), WP2 (pKM101)

Mammalian metabolic system: S9 derived

X	Induced		Aroclor 1254	X	Rat	X	Liver
	Non-induced	X	Phenobarbitol		Mouse		Lung
			None		Hamster		Other
		X	Other β-naphthoflavone		Other		

The S9 fractions were produced by dilution of the liver homogenate with KCl solution (1+3 parts) followed by centrifugation. Aliquots of the supernatant were stored at -80°C in ampoules (small numbers of ampoules could be stored at -20°C for up to a week). An appropriate quantity of S9 supernatant was thawed and mixed with S9 cofactor solution (10% S9 supernatant in the S9 mix). Cofactors were added to reach the following concentrations in the S9 mix:

8 mM $MgCl_2$

33 mM KCl

5 mM Glucose-6-phosphate

4 mM NADP

N 100 mM sodium-ortho-phosphate buffer, pH7.4.

During the experiment, the S9 mix was stored in an ice bath. The S9 mix preparation was performed according to ***Ames et al, 1977***.

Test organisms:

S. typhimurium strains

	TA97	X	TA98	X	TA100		TA102		TA104
X	TA1535	X	TA1537		TA1538		list any others		

E. coli strains

X	WP2 (pKM101)	X	WP2 *uvrA* (pKM101)						

Properly maintained?	X	Yes		No
Checked for appropriate genetic markers (*rfa* mutation, R factor)?	X	Yes		No

Test compound concentrations used:

Experiment I (non-activated and activated conditions): 5000, 2500, 1000, 333, 100, 33, 10 and 3 µg/plate

Experiment II (non-activated and activated conditions): 5000, 2500, 1000, 333, 100 and 33 µg/plate

For all strains triplicate plates were used for all test substance, negative, solvent and positive control treatments.

Study Design and Methods:

Experimental dates: Start: 20 March 2013 End: 16 April 2013

TEST PERFORMANCE

Preliminary Cytotoxicity Assay: Not performed.

Type of Bacterial assay:

- X standard plate test (both experiments –S9, +S9)
- X pre-incubation (60 minutes) (both experiments –S9, +S9)
- __ "Prival" modification (i.e. azo-reduction method)
- __ spot test
- __ other

Protocol:

Bacterial cultures were prepared from frozen stocks by incubating for 4 hours at 37°C in a shaking water bath.

The following materials were mixed in a test tube and poured onto the selective agar plates:

- 100 µL Test solution at each dose level, solvent and positive controls;
- 500 µL S9 mix (+ metabolic activation) or phosphate buffer (- metabolic activation);
- 100 µL Bacteria suspension;
- 2 mL Overlay agar (containing 10.5 mg L-HistidinexHClxH_2O and 12.2 mg Biotin for *Salmonella* strains or 10.2 mg tryptophan for *E.* coli strains).

In the pre-incubation assay 100 µL test solution (solvent or positive control), 500 µL S9 mix/S9 substitution buffer and 100 µL bacterial suspension were mixed in a test tube and incubated at 37°C for 60 minutes. After pre-incubation 2.0 mL overlay agar was added to each tube. The mixture was poured on minimal agar plates.

After the agar was set the plates were incubated upside down for 3 days at 37° C in the dark and then at 4° C until counted.

Following incubation all plates were counted using the Petri Viewer MK2 (Perceptive Instruments Ltd) linked to the Ames Study Manager data collection and analysis system.

Statistical analysis: None – see Evaluation Criteria below.

Evaluation criteria: A positive response in a (valid) individual experiment is achieved when there is a biologically relevant increase in the number of revertants exceeding the threshold of twice the colony count of the corresponding solvent control.

A concentration dependent increase is considered biologically relevant if the threshold is exceeded at one or more concentrations.

An increase exceeding the threshold at only one concentration is judged as biologically relevant if reproduced in an independent second experiment.

A concentration dependent increase in the number of revertant colonies below the threshold is regarded as an indication of mutagenic potential if reproduced in an independent second experiment. However, whenever the colony counts remain within the historical range of negative and solvent controls such an increase is considered not to be biologically relevant.

REPORTED RESULTS

Preliminary cytotoxicity assay: Not performed. The pre-experiment was reported as the main experiment I since the criteria for acceptability were met.

Mutagenicity assay: The plates incubated with mesotrione showed normal background growth up to the limit concentration of 5000 µg/plate in the presence and absence of mammalian metabolic activation in both independent experiments.

No cytotoxic effects, evident as a reduction in the number of revertants occurred in the test groups in the presence and absence of mammalian metabolic activation in both independent experiments.

No increase in revertant colony numbers of any of the six tester strains was observed following treatment with mesotrione at any concentration level, in the presence or absence of mammalian metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance. Appropriate reference mutagens were used as positive controls and showed a distinct increase of induced revertant colonies.

CONCLUSION: There was no evidence of induced mutant colonies over background. Mesotrione did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used. Mesotrione is considered to be non-mutagenic in the *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay.

(Sokolowski A, 2013)

Mesotrione triggers a photomutagenicity study according to the criteria laid down in **Commission Regulation (EU) No 283/2013**. However, such a study has not been conducted with mesotrione because:

- Mesotrione was not genotoxic when tested in 3 *in vitro* studies and one *in vivo* study, reported in **Mesotrione (ZA1296) DAR Volume 3, December 1999** and, more recently, the new technical material has been confirmed to be without activity in the Ames assay.

- Results from the phototoxicity study confirm that mesotrione is not phototoxic. The mechanism for photomutagenicity is assumed to be the same as that for phototoxicity, hence the lack of activity in the phototoxicity study supports the view that mesotrione would be inactive in a photomutagenicity assay.

    - This position is in agreement of that of a recent report by the International Workshop on Genotoxicity Testing Working Group (IWGT)[3] who concluded that data from photogenotoxicity assays provided no added value over and above that available from phototoxicity studies

- Exposure of the skin, either directly following accidental spillage of product, or indirectly via potential systemic exposure, would be very low:

    - Following accidental dermal exposure to the spray dilution of a product containing mesotrione, only a very small percentage of the mesotrione (3%) is predicted to be absorbed (see MCP Section 7). Of this, an even smaller percentage (<0.1%) might be expected to remain within the outer layers of skin.

    - Mesotrione is rapidly absorbed and excreted when dosed orally. It has been shown to be present at higher concentrations than in the plasma in the liver and kidney only, where it reversibly binds to HPPD. The whole body autoradiography study reported in **Mesotrione (ZA1296) DAR Volume 3, December 1999** confirms that mesotrione does not concentrate in the skin after systemic dosing.

- The skin is not a target organ for mesotrione. In rats ocular toxicity (corneal opacity) is observed but this has been shown to be attributable to the severe tyrosinaemia seen in rats as a result of the Mode of Action (MOA) of mesotrione and not to be a direct effect of mesotrione on the eye. The severity of the tyrosinaemia seen in rats is not relevant for human risk assessment; this is discussed in detail in Lewis and Botham (2013)[4]

Syngenta believes that, on the basis of the facts presented above, mesotrione would not present an undue risk to light exposed skin and that a photomutagenicity study is not necessary.

[3] Lynch AM *et al*(2013) Considerations on photochemical genotoxicity II: Report of the 2009 International Workshop on Genotoxicity Testing Working Group Mutation Research 723 91-100

[4] 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. Critical Reviews in Toxicology **43(3**) 185-199 **referenced in MCA Section 9 for toxicology (relevant literature) and summarised in the Literature Section within CA 5.8. (page 51)**

Therefore although mesotrione would trigger a photomutagenicity study based on the criteria laid down in **Commission Regulation (EU) No 283/2013**, Syngenta believes that such a study is not necessary. In addition, there is no published OECD guideline (or indeed any other internationally recognised guideline) for photomutagenicity testing and in the absence of a clear guideline we believe that it is inappropriate to conduct a study at this time.

CA 5.4.2 *In vivo* studies in somatic cells

Please refer to original EU review. No new data or assessment is provided.

CA 5.4.3 *In vivo* studies in germ cells

Please refer to original EU review. No new data or assessment is provided.

CA 5.5 Long-Term Toxicity and Carcinogenicity

Please refer to original EU review. No new data or assessment is provided.

CA 5.6 Reproductive Toxicity

CA 5.6.1 Generational studies

Please refer to original EU review. No new data or assessment is provided.

CA 5.6.2 Developmental toxicity studies

Please refer to original EU review. No new data or assessment is provided.

CA 5.7 Neurotoxicity Studies

CA 5.7.1 Neurotoxicity studies in rodents

Please refer to original EU review. No new data or assessment is provided.

CA 5.7.2 Delayed polyneuropathy studies

Please refer to original EU review. No new data or assessment is provided.

CA 5.8 Other Toxicological Studies

CA 5.8.1 Toxicity studies of metabolites

Please refer to original EU review. No new data or assessment is provided.

CA 5.8.2 Supplementary studies on the active substance

(a)Studies on absorption, distribution, excretion and metabolism in a second species

Please refer to original EU review and CA 5.1 for details of ADME studies in the mouse. These studies were conducted as part of the investigation of the clear species difference in toxicity between the rat and the mouse and to support the use of the mouse as the key species for the human risk assessment of mesotrione.

(b)Studies on the immunotoxicological potential

A review of the currently available toxicity studies on mesotrione has been undertaken and endpoints considered relevant for the identification of potential immunotoxicity have been evaluated.

Report:	KCA 5.8.2/01 Akkan Z and Botham J (2013). Mesotrione –Position Statement Concerning Immunotoxicity Potential. Syngenta Ltd. Jealott's Hill International Research, Bracknell, Berks RG42 6EY. Report No. TK0177144, 22 October 2013. Unpublished. Syngenta File No. ZA1296_10170

A detailed review of parameters related to immune function has been conducted on the existing toxicity database for mesotrione. Repeated-dose studies in rats, mice and dogs were reviewed for any treatment-related changes in a variety of indicators of potential immunotoxicity including leukocyte counts, lymphocyte counts, globulin concentration, macroscopic findings (lymph nodes, thymus, and spleen), organ weights (spleen and thymus), and microscopic findings (bone marrow, lymph nodes, spleen, and thymus).

A thorough review of the toxicology database for mesotrione has shown no evidence of adverse effects on the immune system in rats, mice or dogs. In addition, mesotrione does not belong to a class of chemicals (e.g., the organotins, heavy metals, or halogenated aromatic hydrocarbons) that would be expected to be immunotoxic.

CA 5.8.3 Endocrine disrupting properties

A review of the all relevant data for potential endocrine disruption in mammalian species has been undertaken to fulfil this new data requirement.

Report:	KCA 5.8.3/01 Green R (2013). Mesotrione – Review for Potential for Endocrine Disruption in Mammalian Species Amendment 1. Syngenta Ltd. Jealott's Hill International Research, Bracknell, Berks RG42 6EY. Report No. TK0177145, 29 November 2013. Unpublished. Syngenta File No. ZA1296_10166

This report reviews and summarises all of the relevant available data, including open scientific literature, on mesotrione for potential for endocrine disruption in mammalian species using a weight of evidence approach proposed by the European Chemical Industry Council (CEFIC) Endocrine Modulators Steering Group (EMSG), structured according to the OECD Conceptual Framework (CF) for Testing and Assessment of Endocrine Disrupters.

The relevant data from regulatory studies for mesotrione covered a range of study types including subacute, subchronic, chronic, developmental and reproductive toxicity studies in a range of mammalian species including rat, mouse, dog and rabbit. These studies fell within Levels 1, 4 and 5 of the OECD CF, which address any potentially adverse effect on endocrine relevant endpoints. No relevant data was found in the open scientific literature following a series of comprehensive searches. No *in vitro* or in *vivo* mechanistic studies were available from Levels 2 or 3 of the OECD CF. Each study was evaluated following the CEFIC weight of evidence approach and was assigned a "study significance" score. This was then incorporated into a general descriptive weight of evidence evaluation.

Following, evaluation of each of the relevant studies individually and a subsequent weight of evidence evaluation, it can be concluded that there is no evidence that mesotrione has any potential to interact with the mammalian endocrine system.

Relevant Literature on Other Toxicological Studies

A scientifically peer-reviewed paper considered relevant is detailed below

Reference	Categorisation and Comments	Summary
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. Critical Reviews in Toxicology **43(3**) 185-199	This paper reviews data previously evaluated in Mesotrione (ZA1296) DAR, Volume 3 and uses this as the basis of a systematic analysis of the Mode of Action (MOA) of mesotrione in animals. This is then used as a basis of selecting toxicological endpoints relevant to human risk assessment using the methods established by an ILSI-RSI workshop and discussed in Meek et al (2003)[5] and Seed et al (2005)[6] All studies referenced are considered reliable based on previous review. No new data are presented,	The mode of action (MoA) of the herbicide mesotrione has been empirically established in experimental animals. In this review, we evaluate this MoA and the relevance of this MoA to humans against accepted scientific criteria. The key events in the MoA involve inhibition of the enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD), the second enzyme in the tyrosine catabolic pathway, resulting in excess plasma tyrosine (tyrosinemia). When HPPD is completely inhibited, the clearance of excess tyrosine is dependent upon catabolism by the first and rate limiting enzyme in the catabolic pathway, tyrosine aminotransferase (TAT) and elimination of the products of this catabolism via the urine. The inherent activity of TAT is low in rats and hence they catabolize tyrosine slowly and accumulate tyrosine to very high concentrations in plasma which results in a spectrum of adverse effects that are related to excess tyrosine. There is a large database showing a positive correlation between a range of biological endpoints and elevations in plasma tyrosine. Evidence is presented that clearly establishes a MoA involving tyrosine. Although plausible in humans, the extent and duration of plasma tyrosine elevation in humans is not sufficient to cause adverse effects resulting from the intended use of this herbicide.

A full reliability assessment as described in MCA Section 9 has not been undertaken for this reference as data/studies on mesotrione referenced in this review have been evaluated as part of **Mesotrione (ZA1296) DAR, Volume 3**

[5] Meek ME et al (2003) A framework for human relevance analysis on information on carcinogenic modes of action Crit. Rev. Toxicol. 33 591-654

[6] Seed et al 92005) Overview: using mode of action and life stage information to evaluate human relevance of animal toxicity data. Crit. Rev. Toxicol. 35 663-72

CA 5.9 Medical Data

CA 5.9.1 Medical surveillance on manufacturing plant personnel and monitoring studies

Mesotrione was first handled in 1998 in the research facilities in Richmond, California. A pilot-scale plant was introduced into Cold Creek, Alabama in 2000. Formulation activities have taken place at Syngenta sites in Omaha, Nebraska, St. Gabriel, Louisiana and with a 3rd party toller in the UK. Large scale manufacture is now based in Monthey, Switzerland. Since 2000, the Occupational Health group of Syngenta has maintained a database of incidents involving chemical exposure of workers. A query of the Syngenta internal database in November 2013 for mesotrione resulted in **zero** records of adverse health reported from the handling of mesotrione during synthesis and formulation activities. An earlier report from the legacy Zeneca company gave brief details of one case where a field trials worker spilled some undiluted product onto his leg and developed a rash. This resolved with the standard medical treatment and has not recurred.

CONTROL STRATEGY

The principles of good occupational hygiene practice set a clear hierarchy of control which places primacy to removing the hazard or controlling it by engineering or procedural means, before the use of personal protective equipment (PPE) and respiratory protective equipment (RPE).

This hierarchy of control is clearly followed in Syngenta and includes consideration of aspects such as design and construction of the plant, the cleanliness of the workplace and equipment, working practices and personal hygiene.

For exposure to any substance that can be hazardous by ingestion, absorption or inhalation control must be to a standard that eliminates any health effects.

Ingestion: Eating and drinking are forbidden in areas where chemical handling takes place.

Skin contact: The plant design aims to contain, as far as is possible, chemical exposure by use of total or partial enclosure. Suitable PPE is worn by operators where there is potential for skin exposure.

Inhalation: The plant design aims to contain, as far as is possible, chemical exposure by use of total or partial enclosure and appropriate extraction systems. The plant is designed using the Occupational Exposure Limits (OEL) (see below).

ATMOSPHERIC EXPOSURE STANDARD

Occupational Exposure Limits (OELs) are used in pesticide manufacture as a means of monitoring and controlling atmospheric exposure to chemicals during active ingredient synthesis and formulation. The standards are set by the Syngenta OEL Panel as a primary mechanism of control. These are acceptable concentrations in work-place air based on available toxicology data with the application of a suitable safety factor when making the extrapolation from animal data to a human standard.

The OEL Panel considers the toxicology data available from the package of registration studies together with worker experience during the research, development and commercial manufacturing operations. The standard value is kept under review and may be amended in the light of significant new toxicology or hygiene information.

The current Syngenta OEL value for mesotrione is 10 mg/m3 for an 8-hour time weighted average (TWA) exposure. This value is based on the no-observed effect level (NOEL) of 56 mg/kg bw/day from the 1-year feeding study in mouse, assuming a body weight of 70 kg for an adult worker and a shift inhalation volume of 10m3. The derived value was reduced to the current maximum OEL for nuisance dusts.

In conclusion, mesotrione has been handled in large quantities for over 13 years, at a number of manufacturing and formulation sites and with the use of appropriate control strategies, no adverse health effects associated with the material have been reported in the workforce.

CA 5.9.2 Data collected on humans

Please refer to original EU review. No new data or assessment is provided.

CA 5.9.3 Direct observations

Syngenta has kept detailed records of exposure and poisoning incidences on marketed products for many years. Incident data in Syngenta are collected in two different databases. Reports on cases reported in the USA and Canada are collected in the Prosar database, all other cases are reported into the Adverse Health Incident Database (AHI-DB).

A review of the exposure incidences of mesotrione formulations that have occurred between 2004 and 2012 has been conducted and is presented in the tables below.

52 cases have been recorded in total during this 9 year period of which 37 cases of occupational or accidental, 2 uncertain and 13 cases of intentional exposure related to Mesotrione have been recorded in in total in both databases.

Exposure happened through the dermal, oral, ocular, respiratory and unknown route.

The majority of reported cases were related to incidents leading to none or minor health effects (40 cases). Another 11 cases were assigned to be of moderate severity. The highest severity rate was rated severe, reported for just one incident.

This case was caused by deliberate ingestion. The patient recovered with still on-going health problems at the time of report submission. However, the causal link to the product could not be confirmed. Other suicide attempts were leading to health effects rated to be of lower severity grade. 5 cases of moderate, 6 of minor and 1 of none severity grade have been reported.

Occupational exposures were predominantly leading to exposures without and with minor health effects (8 and 16 cases respectively). 4 of the occupational caused cases were reported to be of moderate severity grade and are considered unlikely to be linked to the active ingredient. Accidental cases resulted to symptoms of minor or none severity grade only.

The following cases of exposure related to mesotrione have been recorded in the PROSAR database (USA/Canada):

Suspected route of exposure	Incidence Type	Severity Grade	Total
Dermal	Occupational	Minor	2
Dermal	Occupational	Moderate	2

Dermal	Occupational	None	4
Dermal	Unknown	Moderate	1
Ingestion	Occupational	None	3
Ocular	Occupational	Minor	1
Unknown	Accidental	Minor	1
Unknown	Uncertain	Moderate	1
Total			**15**

The following cases of exposure related to mesotrione have been recorded in AHI-DB7:

Suspected route of exposure	**Incident Type**	**Severity Grade**	**Total**
Eye	Accidental	minor	1
Eye	Occupational	minor	2
Ingestion	Accidental	minor	1
Ingestion	Accidental	none	2
Ingestion	Accidental	No information	2
Ingestion	Intentional	moderate	5
Ingestion	Intentional	minor	6
Ingestion	Intentional	none	1
Ingestion	Intentional	severe	1
Ingestion	Occupational	minor	1
Ingestion	Occupational	none	1
Inhalation	Occupational	moderate	1
Inhalation	Occupational	minor	4
Skin	Occupational	moderate	1
Skin	Occupational	minor	5
Unknown	Occupational	minor	1
Unknown	Accidental	No information	1
Unknown	Accidental	minor	1
Grand Total			**37**

[7] Countries included in AHI-DB: Albania, India, Algeria, Argentina, Armenia, Australia, Azerbaijan, Belarus, Belgium, Bosnia-Herzegovina, Brazil, Bulgaria, Canada, Chile, China, Colombia, Costa Rica, Croatia, Cuba, Czech Republic, Denmark, Ecuador, Egypt, El Salvador, Fiji, France, Georgia, Germany, Greece, Guatemala, Hungary, India, Indonesia, Iraq, Ireland, Italy, Japan, Jordan, Kazakhstan, Kenia, Kenya, Korea Republic of, Kosovo, Kuwait, Kyrgyzstan, Lebanon, Lithuania, Macedonia, Malawi, Malaysia, Mauritius, Mexico, Moldova, Morocco, Mozambique, New Zealand, Nicaragua, Oman, Pakistan, Peru, Philippines, Poland, Portugal, Qatar, Romania, Russian Federation, Serbia, Slovakia, Singapore, Slovenia, Spain, Switzerland, Syrian Arab Republic, Taiwan, Tajikistan, Thailand, Turkey, Turkmanistan, Ukraine, United Arab Republic, United Kingdom, USA, Uzbekistan, Venezuela, Vietnam, Yemen, Zimbabwe

CA 5.9.4 Epidemiological studies

The company has performed no epidemiological study. The public literature does not report on investigations indicating health effects on the general population due to exposure to mesotrione.

CA 5.9.5 Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical tests

Please refer to original EU review. No new data or assessment is provided.

CA 5.9.6 Proposed treatment: first aid measures, antidotes, medical treatment

Please refer to original EU review. No new data or assessment is provided.

CA 5.9.7 Expected effects of poisoning

Please refer to original EU review. No new data or assessment is provided.

Relevant Literature on Expected Effects of Poisoning

A scientifically peer-reviewed paper considered relevant is detailed below:

Reference	Categorisation and Comments	Summary
Boels D, Monteil-Ganiere C, Turcant A, Bretaudeau M and Harry P (2013). Triketone toxicity: A report on two cases of sulcotrione poisoning. Human and Experimental Toxicology **32(7)** 778-782	Human data therefore considered relevant. Reliability review considered inappropriate as this is not a protocolled study. Data considered relevant for mesotrione as sulcotrione is a herbicide from the same class of chemistry, has the same mode of action and is of similar acute toxicity in animals.	Sulcotrione is a triketone herbicide – this is the first case of poisoning following deliberate ingestion of a compound in the triketone family. Two attempted suicides are reported. Case 1: 90g sulcotrione and alcohol was ingested.. Symptoms: vomiting – all other parameters normal. Plasma sulcotrione 530mg/L after 3 hours/ 310 mg/L after 10 hours. Case 2: Sulcotrione (amount not specified) and a chlorophenoxy herbicide. Symptoms vomiting and others characteristic of chlorophenoxy herbicides. Plasma sulcotrione 550 mg/L after 3 hours, tyrosine elevated (303 umol/L) after 18 hours. Recovery in both cases uneventful – no evidence of ocular effects. Paper concludes that case reports are consistent with animal toxicology of triketones – particularly their relative safety following acute poisoning.

A full reliability assessment as described in MCA Section 9 has not been undertaken for this reference. The data are taken from cases of human poisoning and a reliability review based on Klimisch criteria is, therefore, inappropriate. The data are considered relevant and reliable.