Australian Pesticides &
Veterinary Medicines Authority

The reconsideration of approvals of the active constituent 2,4-D, registrations of products containing 2,4-D and their associated labels.

Preliminary Review Findings (Environment)

Part 1:

2,4-D Esters

Volume 2: Technical Report

Appendix II- 2,4-D Esters

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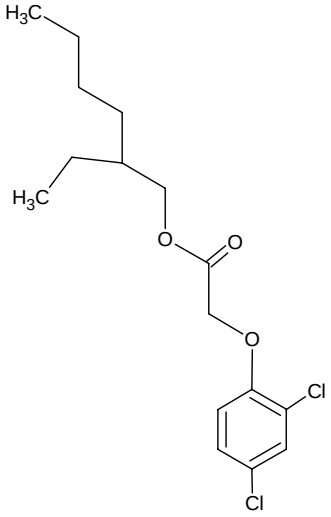
Australian Pesticides &
Veterinary Medicines Authority

Canberra
Australia

APPENDIX II – Technical Report for Esters of 2,4-D

Identity, Physical and Chemical Properties, 2,4-D Ethylhexyl ester

	Ethylhexyl ester
Common name (ISO)	2,4-D 2-EHE
Chemical name (IUPAC)	(2,4-dichlorophenoxy) acetic acid, 2-ethylhexyl ester
Chemical name (CA)	-
CAS No	1928-43-4
EEC No	217-673-3
Minimum purity[1]	920 g/kg minimum as given by FAO specification
Molecular formula	$C_{16}H_{22}Cl_2O_3$
Molecular mass	333.26
Structural formula	
Melting point[1]	<-37°C
Boiling point[1]	not determinable. Degradation occurs at >200°C
Appearance[1]	non viscous clear golden yellow liquid, characteristic odour of aromatic esters.
Relative density[1]	pure a.s.: 1.1502 technical a.s.: 1.1527
Vapour pressure[2]	4.80×10^{-4} Pa at 25°C
Henry's law constant[3]	$1.82X10^{-5}$ atm.m/mole
Solubility in water[4, 5]	86.7 µg/L (pH effect not investigated as, the compound is neither acidic nor basic). 32.4±3.2 µg/L in industrial water.
Partition co-efficient (log Kow)[6]	logKow= 5.78 at 25°C (pH not stated)
Dissociation constant	not applicable.

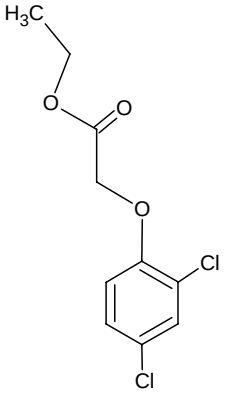
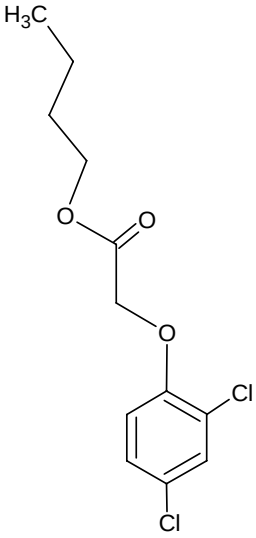
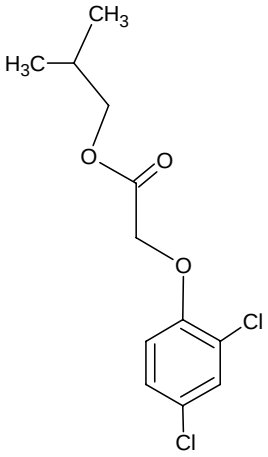
1) EC, 2001; 2) Chakrabarti and Gennrich, 1987b; 3) HenryWin v3.10; 4) Helmer, 1987(a); 5) Potter, 1990; 6) Helmer, 1987b.

With one minor exception, all data received by the APVMA for 2,4-D esters related to the ethylhexyl ester as described above.

Section 4.2 in the Overview Report outlines the application rates and use patterns based on a review of 2,4-D labels registered in Australia. Where esters are the 2,4-D form registered, 33 product labels were identified, and 6 of these use the EHE form. The other 20 products used 2,4-D in its ethyl ester (13 products), butyl ester (1 product) or isobutyl ester (6 products) forms, with 13 products using a mixture of

ethyl and butyl esters. No data have been received for reviewing these shorter chain esters except for a hydrolysis study with the butyl ester described further below.

The following table provides some of the basic physico-chemical information for these three esters. For clarity, it is important to consider the results in conjunction with the comments following the table.

Common name:	2,4-D EE	2,4-D BE	2,4-D IBE
Chemical name:	2,4-D Ethyl Ester	2,4-D Butyl Ester	2,4-D Isobutyl ester
Molecular formula:	$C_{10}H_{10}Cl_2O_3$	$C_{12}H_{14}Cl_2O_3$	$C_{12}H_{14}Cl_2O_3$
CAS Number:	533-23-3	94-80-4	1713-15-1
Molecular weight:	249.1	277.15	277.15
Structure:			
Vapor pressure (20°C):	$1.1X10^{-3}$ mmHg	$6.16\ X10^{-5}$ mmHg; $3.97\ X10^{-4}$ mmHg	$1.2\ X10^{-4}$ mmHg
Henry's Law:	$3.25\ X10^{-7}$ atm.m^3/mole	$4.88\ X10^{-7}$ atm.m^3/mole	$1.25\ X10^{-5}$ atm.m^3/mole
Solubility (25°C):	111 mg/L	46 mg/L	3.5 mg/L
Log K_{ow}:	3.39	4.38	4.30

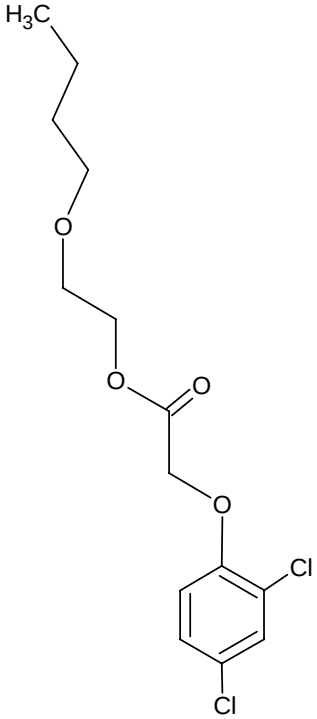
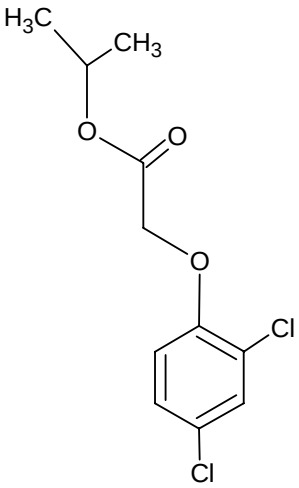
EPISuite was used to calculate all values where experimental data do not exist. Experimental values are available from the US EPA EPISuite experimental database for water solubility and vapour pressure for 2,4-D EE and 2,4-D BE. The vapour pressure result for 2,4-D EE is also reported in WHO (1984 and 1989). However, the vapour pressure result for 2,4-D BE differs between these references. A vapour pressure of $6.16X10^{-5}$ mmHg is reported in the EPISuite experimental database while $3.97X10^{-4}$ mmHg is reported in WHO 1984 and 1989.

No experimental data are available for 2,4-D IBE. All values reported above have been calculated from EPISuite. It is worth noting that this estimation software tended to underpredict the water solubility for the other two esters by a factor of between around 4 (EE) and 15 (BE) although vapour pressure predictions were relatively close, particularly for the butyl ester.

In any event, these esters are all expected to be moderately volatile to volatile based on the scale of Mensink *et al* (1995). This statement is further supported by German research. Guth *et al* (2004) state that, based on direct measurements, no noticeable volatility can be expected from compounds with a vapour pressure below 10^{-3} Pa from soil and 10^{-4} Pa from crops, and this is fully confirmed by indirect measurements. Converting the above vapour pressures for EE, BE and IBE to Pa gives results of

$1.47X10^{-1}$, $5.29X10^{-2}$ to $8.2X10^{-3}$, and $1.61X10^{-2}$ Pa respectively, indicating they can all be expected to volatilise from soil and crop surfaces. Further, the calculated Henry's Law Constants indicate the three esters may be moderately volatile to volatile from water bodies.

Several data for two other esters apparently commonly used in the United States are available, but not provided to the APVMA for this review. These are 2,4-D butoxyethyl ester (2,4-D BEE) and 2,4-D isopropyl ester (2,4-D IPE). A review of 2,4-D products reveals these esters are not used in Australia (see Section 4.2 of Overview Report). However, data assessed by the US EPA with respect to these esters will be reported in the overview report for completeness, and use for surrogate values if required. Following are the identity and physico-chemical properties of these two esters as described by the US EPA.

Common name:	2,4-D BEE	2,4-D IPE
Chemical name:	2,4-D butoxyethyl ester	2,4-D Isopropyl Ester
Molecular formula:	$C_{14}H_{18}Cl_2O_4$	$C_{11}H_{12}Cl_2O_3$
CAS Number:	1929-73-3	94-11-1
Molecular weight:	321.20	263.12
Structure:		
Vapour pressure (20^oC):	$2.4X10^{-6}$ mmHg @ 25^0C	$5.3X10^{-6}$ mbar ($3.97X10^{-6}$ mmHg)
Henry's Law:	not calculated due to insolubility	6.3 E^{-5} atm-m^3/mole
Solubility (25^oC):	12.7 ± 1 µg/L Based on MRID #41669501	230 mg/L
Log K_{ow}:	4.35	3.81

Vapour Pressure: In addition to the above results, the vapour pressure of the butoxyethyl ester of 2,4-D was measured by the Knudsen-Effusion/Weight Loss method (Chakrabarti, 1989). Testing with two separate temperature ranges of 84-

182°C and 170-300°C and extrapolating the results back to 25°C resulted in vapour pressures of $6.1X10^{-4}$ Pa and $6X10^{-4}$ Pa respectively.

The vapour pressures in the above table convert to $3.2X10^{-4}$ and $5.3X10^{-4}$ Pa for 2,4-D BEE and 2,4-D IPE respectively. These are similar in magnitude to that for 2,4-D EHE, and suggest they are only marginally volatile from soil, but potentially volatile from plant surfaces.

Hydrolysis

In addition to the following 2,4-D 2-EHE data, the US EPA has assessed hydrolysis data for both 2,4-D BEE (1 study) and 2,4-D IPE (2 studies) (MRID numbers 41353701; 41349601 and 43441201). These data have not been provided to the APVMA but will be considered in the overview report.

Test material:	**2,4-D 2-Ethylhexyl Ester**
Report:	Concha *et al*, 1993a
Guidelines:	US-EPA Subdivision N; 161-1
GLP:	yes

Test System

^{14}C-2,4-D 2-EHE, uniformly labelled in the phenyl ring, was tested for its hydrolytic stability at pH 5, 7 and 9 in sterile aqueous solutions containing 1% acetonitrile co-solvent. The study was conducted for up to 30 days at pH 5 and 7 and for 6 days at pH 9 at an approximate application rate of 30 ppb.

Samples were maintained in the dark in an incubator at 25°C during the study period. The analytical methodology involved HPLC analysis for quantitation and product co-chromatography, with reference standards and TLC for confirmation of degradates.

Findings

2,4-D EHE degraded slowly at pH 5 and moderately at pH 7. After 30 days exposure, 77.7%AR was identified as parent material in the pH 5 system. In comparison, only 59.3% 2,4-D EHE remained in the pH 7 samples. The major degradate was 2,4-D which represented 4.2% and 28.2% AR for pH 5 and pH 7 respectively. 2,4-D EHE bound persistently to glass surfaces with passing time at acidic and neutral pH. After 30 days, 7.9% and 5.9%AR was recovered in the base extraction of the vessel walls of the pH 5 and pH 7 samples respectively.

In the pH 9 solutions, 2,4-D EHE degraded rapidly. After 144 hours, this chemical represented 14.2% AR with 2,4-D being the major metabolite formed by cleavage at the ester linkage, and representing 81.4% AR.

The following half lives were obtained:

pH 5		pH 7		pH 9	
$t_{1/2}$	r^2	$t_{1/2}$	r^2	$t_{1/2}$	r^2
99.7 days	0.931	78.3 days	0.929	52.2 hours	0.975

Conclusion

Hydrolysis is expected to be a significant route of dissipation of 2,4-D EHE in basic aqueous environments.

Test material: **2,4-D 2-Ethylhexyl Ester**

Report: Concha *et al*, 1993b

Guidelines: US-EPA Subdivision N; 161-1

GLP: yes

Test System

The hydrolysis of ^{14}C-2,4-D 2-EHE uniformly labelled in the phenyl ring was examined in natural water from the Tittabawasse River in Midland, Michigan, containing 1% acetonitrile co-solvent. The study was conducted for up to 24 hours, at an approximate application rate of 29.5 ppb. Samples were maintained in the dark in an incubator at around 25^{o}C. The analytical methodology involved HPLC analysis for quantitation and product co-chromatography with reference standards and TLC for confirmation of degradates.

The water used had an alkalinity of 164 mg $CaCO_3$/L, total suspended solids of 13 mg/L, a pH of 7.8 and hardness of 204 mg $CaCO_3$/L. Microbial viability of the 0 and 24 h samples was established by duplicate plating of samples in Tripticase Soy Agar (bacteria). The culture plates were incubated at 35^{o}C for 2 days to detect aerobic bacteria and observed after 48 hours. Heavy growth was detected on all plates after 2 days incubation indicating that the microbial activity of the test system was preserved throughout the study period.

Findings

In this natural water, 2,4-D EHE degraded rapidly. After 24 hours of exposure, 7.2% AR was present as parent. The only major degradates observed at the end of the study was 2,4-D representing 93.8% AR.

The half-life was calculated to be 6.2 hours ($r^2 = 0.997$) based on psuedo first order kinetics.

Conclusion

This half-life in this study is considerably faster than those observed in sterile buffer systems described in Concha *et al* 1993a above and indicates hydrolysis is a major dissipation method for this chemical in natural aqueous environments.

Test material: **2,4-D 2-Ethylhexyl Ester**

Report: Concha *et al*, 1993c

Guidelines: US-EPA Subdivision N; 161-1

GLP: yes

Test System

The hydrolysis of ^{14}C-2,4-D 2-EHE uniformly labelled in the phenyl ring was examined in Catlin silty clay and Hanford sandy loam soil slurries with the following soil characteristics:

Table A2.1: Soil characteristics

Texture	Origin	% Sand	% Silt	% Clay	% OM	CEC (meq/100 g)	pH	WHC[1]
Silty clay loam	Catlin	11.2	60	28.8	3.87	10.08	6.9	27.16

Sandy loam	Hanford	58.2	30	10.8	0.66	3.78	6.7	10.03

1) % at 1/3 Bar

Soil viability was evaluated by counting total colony-forming units (CFUs) of aerobic bacteria, actinomycetes and fungi. The results showed both soils were viable.

The study was conducted over a 4 hour period with samples maintained in the dark in an incubator at 25^{o}C. For application, an aliquot of stock solution was added to a mixture of the soils and deionised water and the whole mixture was vortexed and placed in the incubator.

The extraction procedure included acidification with aqueous concentrated HCl, then addition of the extraction solvent (ethyl acetate:acetone, 85:15). The sample was vortexed, then shaken. The aqueous layer was separated from the organic layer, combined with the soil, and the extraction procedure repeated once more. The organic layers from both extractions were combined and dried. Aliquots of the organic extract and the aqueous layer were analysed by LSC. The analytical methodology involved HPLC analysis for quantitation and product assignment by co-chromatography with reference standards and TLC for confirmation of degradates.

Findings

Radiocarbon recoveries averaged 102.4±1.4% and 95.5±1.4% for Catlin and Hanford soil slurry samples respectively. Less than 4% AR was unextractable at the end of the study period for both Catlin and Hanford samples.

2,4-D EHE degraded rapidly in both soil slurries with 9.6% and 12.6% AR remaining as parent in the Catlin and Hanford samples respectively at the end of the 4 hour study period. The major degradate observed in both soil slurries was the hydrolysis product 2,4-D. After 4 hours, 2,4-D accounted for 88.3% and 78.6% AR in Catlin and Hanford soil slurries respectively.

The half-lives of 2,4-D EHE were calculated to be 1.25 hours ($r^2 = 0.973$) and 1.45 hours ($r^2 = 0.948$) for the Catlin and Hanford samples respectively.

Conclusion

The half-lives from this study are considerably faster than those for hydrolysis in sterile buffer systems described in Concha *et al* 1993a above, and in natural water as described in Concha *et al*, 1993b above. The results indicate that hydrolysis is a major mode of dissipation in soil/water environments, and that the soil, or the microbiological activity associated with it, leads to enhanced rates of hydrolysis relative to non-soil systems.

Photodegradation in Water

In addition to the following 2,4-D 2-EHE data, the US EPA has assessed photodegradation in water data for 2,4-D BEE (one study – MRID number 41483101). This study has not been provided to the APVMA but will be considered in the overview report.

Test Material: **2,4-D 2-Ethylhexyl Ester**

Report: Concha and Shepler, 1993b.

Guidelines: US-EPA Subdivision N; 161-2

GLP: yes

Test System

[^{14}C]2,4-D 2-EHE, uniformly labelled in the phenyl ring, was exposed to natural sunlight in quartz tubes in an aqueous pH 5 buffer solution for up to 31 days at an application rate of 30.7 ppb, which is below the aqueous solubility of this compound. Dark control samples were run concurrently in pyrex tubes wrapped in aluminium foil to prevent irradiation. The sample tubes were placed in a deionized water bath and the temperature monitored continuously during the study period. The average temperature of the water bath was around 25.3^{o}C for the study period. A PNAP-PYR (*p*-nitroacetophenone-pyridine) chemical actinometer was run concurrently with the samples for the calculation of quantum yield of aqueous photolysis. Probe work indicated no trapping of volatiles was necessary for this study. The analytical methodology involved HPLC for quantitation and product co-chromatography with reference standards. GC/MS and TLC were used for confirmation of degradates.

Findings

Radiocarbon recoveries averaged 98.1±3.4% and 99.5±3.2% AR for light exposed and dark control samples respectively.

Table A2.2 – Aqueous Photolysis of 2,4-D 2-EHE and Formation of its Photodegradation Products

Time (days)	2,4-D 2-EHE	2,4-D	2,4-DCP	ECP[1]	Other	Bound	Total
0	94.8	0.0	0.0	0.0	0.4	5.9	101.1
3	92.2	0.4	0.0	0.2	0.8	5.2	98.8
7	88.9	0.7	0.7	0.4	1.0	10.3	12.0
14	87.0	3.2	0.1	0.8	1.2	4.6	96.9
21	81.9	1.7	4.7	1.4	0.8	5.5	96.0
31	80.3	5.0	2.1	1.1	1.0	5.1	94.6

1. 2-ethylhexyl 4-chloro phenoxyacetate.

Recovery of parent compound in the dark control ranged from 93.2% AR at day 14 to 84.8% AR at day 31.

Conclusion

The half-life of degradation of 2,4-D 2-EHE was calculated to be 128.2 days in the light exposed samples based on pseudo-first order kinetics. The half-life for the dark control samples was calculated to be 252.5 days. The direct quantum yield of 2,4-D-EHE was determined to be 1.246. The results of this study indicate that photolysis is unlikely to be a significant removal mechanism for 2,4-D esters in aqueous environments.

Photodegradation in Soil

No data were provided for this end-point. The US EPA reports a soil photodegradation study for 2,4-D BEE. This study has not been provided to the APVMA but will be considered in the overview report.

Degradation in Soil and Water

Soils – Aerobic

No data were provided for this end-point. The US EPA has assessed an aerobic soil degradation study for 2,4-D IPE (MRID number 43149601). This study has not been provided to the APVMA but will be considered in the overview report.

Soils – Anaerobic

No data were provided for this endpoint.

Water – Aerobic

No data were provided for this end-point. The US EPA has assessed an aquatic aerobic degradation study for 2,4-D IPE (MRID number 43149601). This study has not been provided to the APVMA but will be considered in the overview report.

Water – Anaerobic

No data were provided for this end-point. The US EPA has assessed an anaerobic aquatic degradation study for 2,4-D BEE (MRID number 42574701). This study has not been provided to the APVMA but will be considered in the overview report.

Mobility

Adsorption/Desorption

Test Material: **2,4-D 2-Ethylhexyl Ester; 2,4-D Butyl Ester**

Report: McCoy and Lehman, 1988

Guidelines: Not stated. Appears to correspond to US-EPA Subdivision N; 163-1

GLP: Yes

Test System

The sorption properties of the ring-radiolabelled 2,4-D esters were investigated using four surface soils of varying characteristics as follows:

Table A2.3: Soil characteristics

Texture	Series	% Sand	% Silt	% Clay	OC	CEC (meq/100 g)	pH	% MC
Silt loam	Catlin	16	60	24	2.23	15.0	5.9	16.22
Sandy loam	Hanford	64	26	10	0.22	15.9	7.5	4.65
Loam	Barnes	40	38	22	3.08	5.2	6.8	15.61
Clay	Mahoon	14	40	46	1.26	18.8	7.0	16.76

Prior to the adsorption study, the hydrolysis rate of the 2,4-D esters was determined by analysis of a solution of ester that had been mixed with soil for various time periods. A solution containing 10 mL of 2.1 mg/L radiolabelled 2,4-D 2-EHE ester in 0.01 M $CaCL_2$ was added to 4 g Mahoon soil in a foil covered 25 mL centrifuge tube and placed on a shaker. The tube was removed at 30, 90 and 210 minutes and centrifuged for 15 minutes. The supernatant was analysed by HPLC and duplicate aliquots were also removed for direct LSC analysis. The soil was resuspended and placed back on the shaker.

The hydrolysis rate of the butyl ester was determined in the same manner except the solution contained 2.5 mg radiolabelled 2,4-D butyl ester and the time points were 45, 110 and 175 minutes.

Findings:

Both 2,4-D esters hydrolysed quickly in the Mahoon soil. The half-life for 2,4-D 2-EHE was 79 minutes while the butyl ester was less stable with a half-life of 26 minutes.

Conclusion:

The rapid hydrolysis rates indicate the esters are not stable enough to allow a batch equilibrium study to be performed for determination of K_d values. The study of the ester hydrolysis rate revealed that the esters hydrolysed quickly to the acid in the presence of soil indicating that the behaviour of the ester in the environment can be modelled by using the parameters found for the acid.

Leaching Potential

Column Leaching Studies

No column leaching data were provided for 2,4-D esters.

Aged Column Leaching Studies

No aged column leaching studies were provided for 2,4-D esters.

Lysimeter/Field Leaching Studies

No lysimeter or field leaching studies were provided for 2,4-D esters.

Fate and Behaviour in Air

In addition to the following 2,4-D 2-EHE data, the US EPA has assessed photodegradation in air data for 2,4-D BEE (1 study - MRID number 41483103). This study has not been provided to the APVMA but will be considered in the overview report.

Test Material: **2,4-D 2-Ethylhexyl Ester**

Report: Doyle, 1991

Guidelines: US-EPA Subdivision N; 163-2

GLP: Yes

Test System

The test substance was the formulation Esteron 99 Concentrate mixed with a small quantity of ^{14}C 2,4-D-2EHE. The spray solution contained 1% hexane to simulate the use of fuel oil additives. The formulation contains 66.6% ai (43.7% acid equivalent). Soil used in the study was classified as a sandy loam with the following characteristics:

Texture	% Sand	% Silt	% Clay	% OC	CEC (meq/100 g)	pH	% MC
Sandy loam	78	10	12	0.69	3.2	6.5	4.17

The soil was sieved (2 mm) prior to use and was microbially active. The bulk density was determined to be 1.4 g/cm^3.

Treated soil was immediately placed in a volatility chamber where air at 95-100% relative humidity and 25°C was continuously passed over the soil at air flow rates of 100 and 300 mL/minute. The pressure in the volatility chamber was held at 1.00 atmosphere. Off-gases were passed through sorbent tubes then through KOH traps. These were periodically replaced and analysed during the 15 day test interval.

Several preliminary trials were undertaken. A spray application test was conducted to determine the spray efficiency. A preliminary investigations were performed to determine the length of time the definitive study should run, to evaluate the trapping efficiency of the traps and the establish the schedule for changing the traps.

In the definitive trial, 3 pans of soil were treated sequentially with two sealed into separate volatility chambers and the third used as a control for immediate extraction. The sorbent tubes and KOH traps were replaced after 1, 3, 5, 7, 9, 11, 3 and 15 days in the 100 mL/minute study, and 1.5, 3, 7, 11 and 15 days in the 300 mL/minute study. After 15 days, the pans of soil were removed from the chambers and immediately extracted. The extracts were analysed by HPLC and LSA. The extracted soil was air dried, mixed and analysed by combustion and LSA.

Findings

The actual rates of application (average of control and two treated pans) were 17.7 kg ae/ha in the 100 mL/minute test and 17.8 kg ae/ha in the 300 mL/minute test. The mass balance averaged 98.9% AR and average measured airflow rates were 98 and 286 mL/minute.

As found in the preliminary investigation, volatilisation in the definitive trial was low. Distribution of residues (% AR) is described below:

Table A2.4: Distribution of Soil Applied ^{14}C-residues

	^{14}C-Soil Residues (%)				^{14}C-Volatiles (%)	
Air flow rate (mL/minute)	Replicate	Extracted	Unextracted	Total	Sorbent tubes	KOH Traps
100	1	84.61	15.31	99.92	0.05	0.03
100	2	86.67	13.22	99.89	0.05	0.07
100	Average	85.64	14.27	99.90	0.05	0.05
300	1	89.36	10.37	99.73	0.22	0.05
300	2	82.22	17.50	99.72	0.23	0.05
300	Average	85.79	13.94	99.73	0.22	0.05

The higher flow rate resulted in higher losses through volatility. Volatilisation was most rapid immediately after application. Rates of volatilisation declined with time until day 7. From then until day 15, rates were relatively constant.

The highest losses occurred during the first day. Average volatilisation rates for the 100 and 300 mL/minute air flow rates were $8.06X10^{-4}$ and $3.45X10^{-3}$ μg/cm^3/hour respectively with average air concentrations at these rates of 34.84 and 46.06 μg/m^3 respectively.

HPLC/UV analysis of the soil extracts showed that between 69.5 and 77.5% of the 2,4-D 2-EHE was converted to 2,4-D acid during the study. No other significant ^{14}C-labelled compounds were detected.

Conclusion

The volatilisation of 2,4-D 2-EHE from the light textured, low organic matter soil on which the formulation was applied was low.

Modelled data:

The calculated Henry's Law Constants of $1.82X10^{-4}$, $3.25\ X10^{-7}$, $4.88\ X10^{-7}$, and $1.25\ X10^{-5}$ atm.m^3/mole for the EHE, EE, BE and IBE 2,4-D esters are indicative of at least moderate volatility from water (Mensink *et al*, 1995).

No experimental data for degradation or volatility in the atmosphere were provided. However, the former was considered through modelling. For the four 2,4-D esters (2-EHE, EE, BE and IBE) registered in Australia, the rate constant for reactions of with OH radicals (photochemical oxidative degradation) in the atmosphere was calculated using the AOP program [AOPWIN Program (Atmospheric Oxidation Program for Microsoft Windows 3.1) Version 1.91, provided as part of the US EPA EPIWIN software. First, the rate constant k_{OH} of the various esters were estimated based on the chemical structure. The resulting value was

The half-life of this process is calculated by the following equation:

$$t_{½} = \ln 2/k' = \ln 2/k_{OH}\ X\ [OH\ radicals]$$

The diurnally and seasonally averaged concentration of tropospheric hydroxyl radicals used by the AOP program is $1.5\ X\ 10^6$ cm^{-3}. Outputs from the modelling were as follows based on a 12 h:12 h light:dark day:

Ester	Smiles String	Rate Constant[1]	Half-life (Days)
2,4-D 2-EHE	Clc1cc(Cl)ccc1OCC(=O)OCC(CC)CCCC	14.5058	0.737
2,4-D EE	Clc1cc(Cl)ccc1OCC(=O)OCC	5.261	2.03
2,4-D BE	CCCCOC(=O)COc1ccc(Cl)CC1Cl	8.1666	1.31
2,4-D IBE	Clc(cc(Cl)c1OCC(=O)OCC(C)C)cc1	8.1582	1.31

1) Rate constant, K_{OH} ($X\ 10^{-12}$ cm^3/molecule/second)

This modelling indicates the shorter chain esters have longer atmospheric half-lives than the longer chain EHE. Australia does not have formal persistence criteria for chemicals in air. The Stockholm Convention provides scientifically based criteria for potential POPs, and Annex D of this Convention states with regard to the potential for long-range environmental transport of chemicals that for a chemical that migrates significantly through air, its half-life in air should be greater than 2 days. Further, the US EPA and Environment Canada consider a chemical persistent in air if that chemical has a half-life of 2 days or greater.

Given the longer half-life of the shorter chain 2,4-D esters in air (and in particular, the predicted half-life of 2,4-D EE being greater than 2 days), along with the volatile nature of these compounds, it is apparent they may be susceptible to significant off target movement, and this will be explored further in the risk assessment.

Field Dissipation Studies

Forestry

Test Material: **2,4-D 2-Ethylhexyl Ester**

Report: Barney, 1996.

Guidelines: US-EPA Subdivision N; 164-3

GLP: Yes

Test System

2,4-D 2-EHE has been shown to hydrolyse rapidly to 2,4-D. The objective of this forest field soil dissipation study was to determine the extent and rate of residue dissipation and mobility of 2,4-D and metabolites (applied as 2,4-D 2-EHE) in soil, foliage, leaf litter, sediment and water when applied according to a forest use pattern in the USA. According to the test protocol, the field trial was to be in a commercial-type forest area with brush at least 0.6 m tall. Adjacent to, but not more than 180 m from the terrestrial sampling area in each plot (treated and untreated control – UTC) was to be either a pond or a stream.

The study was conducted near Hawkinsville, Georgia and involved one treated and one non-treated site. The treated field was 130 X 440 feet (around 39.6 X 134 m). This was split into 5 sampling sub-plots of 88 X 130 feet (around 26.8 X 39.6 m). The distance between the treated and untreated fields was 700 feet (around 215 m) and the untreated field was 100 X 300 feet (30.5 X 91.4 m) consisting of 3 sampling sub-plots each 100 X 100 feet (30.5 X 30.5 m). The soil characteristics of the field were as follows:

Table A2.5: Soil and sediment characteristics

Sampling depth	Texture	% Sand	% Silt	% Clay	% OM	CEC (meq/100 g)	pH	% MC
Soil								
0-15 cm	Sandy clay loam	69.2	8.0	22.8	1.60	4.03	5.9	10.45
15-30 cm	Sandy clay loam	59.2	12.0	28.8	0.85	3.53	5.9	11.84
30-45 cm	Sandy loam	73.2	8.0	18.8	0.32	1.87	6.2	8.12
Sediment (in the 0-5 cm layer)								
Treated	Sandy clay	47.2	16.0	36.8	4.05	10.51	5.8	25.52
Untreated control	Clay	3.2	32.0	64.8	3.78	10.62	5.4	36.32

The soil had a bulk density of 1.38 g/cm^3 in the top 15 cm layer. The sediment in the treated plot had a bulk density of 1.13 g/cm^3 while the untreated plot sediment had a bulk density of 1.23 g/cm^3.

Prior to application, the experimental site area was prepared by disking two strips of exposed soil in the treated plot and one strip in the untreated control plot. The chemical was applied in the formulated product, Esteron 99C using an Air Tractor 301 Airplane. At application the boom was around 5.2-5.8 m above the ground. Application rates were verified using monitoring pads. The application rate was a nominal 4.5 kg ae/ha.

Soil, sediment, foliage and leaf litter samples were assayed for residues of 2,4-D-2-EHE, 2,4-D acid, 2,4-DCP and 2,4-DCA. The above analytes as well as 4-chlorophenoxy acetic acid (4-CPA) and 4-chlorophenol (4-CP) were also analysed in water.

Soil samples from exposed and protected plots on days –1, 0, 1, 3, 7, 15, 30, 77, 91, 119, 180 and 359. Samples were collected to depths of 45 cm and sectioned into 15 cm segments. On day 0, only the top 15 cm was sampled. Exposed soil was soil exposed to the test substance application and not protected by leaf litter or foliage. Protected soil was soil covered by overlying leaf litter during the application.

Foliage samples (Long leaf pine foliage – *Pinus palustris*) was sampled on the same schedule as outlined for soil above. Leaf litter was sampled in locations exposed to application also on the same schedule as the soil sampling. Water samples were

collected on days –1, 0, 1, 7 and 30 following application. Samples were collected from the top 5 cm and bottom 15 cm from both the treated and UTC ponds. Sediment samples were collected from the 0-5 cm layer in both ponds following the water sampling schedule, with additional samples collected on days 91 and 180 after application.

To determine the stability of the analytes throughout the chain-of-custody, field spikes of soil cores, foliage, leaf litter, sediment and water fortifications were prepared in duplicate for each analyte.

Findings:

Precipitation over the course of the study was around 107% of normal. The three months leading up to application, however, were significantly drier than the 30 year average. The first three months following application were about the 30 year average.

Meteorological data provided in the study report showed 0.38 inches (9.6 mm) of rain falling on the day of application with 0.53 (13.5 mm) and 1.21 (30.7 mm) falling on days 2 and 3 following application respectively.

Table A2.6: Residues of 2,4-D 2-EHE and 2,4-D (ppm) in four tested media.

Medium	Analyte[1]	-1	0	1	3	7	15	30	77[2]	91
Exposed soil	2-EHE	-	0.141	-	0.029	-	-	-	-	-
	2,4-D	-	0.145	-	0.074	-	0.003	0.003	-	-
Protected soil	2-EHE	-	0.058	0.041	0.008	0.003	-	-	-	-
	2,4-D	-	0.107	0.203	0.013	0.005	0.008	-	-	-
Foliage	2-EHE	-	36.94	15.52	11.09	0.419	2.48	0.239	0.042	-
	2,4-D	-	43.01	31.46	25.03	4.32	7.01	1.73	0.897	0.331
Leaf litter	2-EHE	-	50.57	12.07	13.93	11.10	1.018	1.32	0.311	0.598
	2,4-D	-	30.61	23.73	18.43	15.92	1.51	1.90	0.473	1.067

1 LOQ = 0.01 ppm for soil and leaf litter; 0.1 ppm for foliage.
2 62 days for foliage and leaf litter sampling.

In exposed soils, no residues of 2,4-D 2-EHE were detected in any samples taken from day 7 onwards after the aerial application while 2,4-D was not detected from Day 77 onwards. In protected soils, 2,4-D 2-EHE decreased to a mean of 0.003 ppm in 7 days after treatment (DAT) sample with no further residues found after this time. 2,4-D decreased to a mean of 0.008 ppm in 15 DAT samples with no further detections after this time.

In the foliage samples, 2,4-D 2-EHE residues generally declined to a mean of 0.239 ppm at the 30 day sampling interval and then to a mean <LOQ after 62 DAT. Residues of 2,4-D declined to a mean of 0.325 ppm at the 180 DAT sampling interval and were <LOQ at study end. Residues of 2,4-DCP were found at 0.339 ppm at 0 DAT where they were found at around the same level until 3 DAT. From there, they generally declined (a second peak of 0.229 ppm was found at 30 DAT) with 0.081 ppm found at 118 DAT and no further residues detected thereafter. 2,4-DCA was detected sporadically with 0.159 ppm found at 7 DAT (detected in all three replicates) and 0.07 ppm at 180 DAT (found in 2 of the three replicates).

Residue decline in leaf litter was slower than the other media with residues of all analytes still being detected at the slower end. Table A2.6 above shows residues of 2,4-D 2-EHE and 2,4-D up until 91 DAT. Residues of 2,4-D 2-EHE found at days 118, 180 and 359 were 0.254, 0.368 and 0.116 ppm respectively (mean of three replicates), while those for 2,4-D were 0.265, 0.433 and 0.179 respectively (mean of

three replicates). Residues of 2,4-DCP were highest at 0 DAT (2.9 ppm) with 2.22 ppm found at 7 DAT. From this time point, 2,4-DCP was found at 0.2 ppm or less and had declined to be 0.036 ppm at 359 DAT. 2,4-DCA was found at every sampling point and was above 0.2 ppm at Day 0 until its peak of 0.37 ppm at 7 DAT. From there, the decline was slow with 0.062 ppm still being found at 180 DAT. By study end, this analyte was found at 0.01 ppm.

Despite the relatively high combined rainfall of days 2 and 3, water and sediment samples collected from a pond down-slope of the treated plot showed no detection of any analyte tested for at any of the sampling times suggesting there was no run-off of the chemical.

The half-lives calculated by the authors for the various metabolites are shown in Table A2.7 below. They were calculated using least squares linear regression using the LINEST function in Microsoft Excel, version 5, spreadsheet software. Half-lives were calculated for dissipation curves using the slope derived from the regression model in the equation T1/2 = -ln(2)/m.

Table A2.7: Half-Life Values from Linear Regression for Residues in Forestry Matrices

Medium	Analyte	Slope (m)	Half-life (days)	Coefficient of Determination (r^2)
Exposed soil	2,4-D 2-EHE	-0.7047	1.0[1]	0.3684
	2,4-D	-0.1750	4.0[2]	0.6097
Protected soil	2,4-D 2-EHE	-0.4178	1.7[3]	0.9176
	2,4-D	-0.1951	3.6[4]	0.5118
Foliage	2,4-D 2-EHE	-0.0963	7.2[5]	0.7517
	2,4-D	-0.0295	23.5[6]	0.7311
	2,4-DCP	-0.0158	44.0[7]	0.5637
Leaf litter	2,4-D 2-EHE	-0.0136	51.0[8]	0.5454
	2,4-D	-0.0133	52.2[8]	0.5665
	2,4-DCP	-0.0101	68.3[8]	0.5029
	2,4-DCA	-0.0082	84.7[8]	0.7349

1 Calculated from day 0 – 3
2 Calculated from day 0 – 30
3 Calculated from day 0 – 7
4 Calculated from day 0 – 15
5 Calculated from day 0 – 62
6 Calculated from day 0 – 180
7 Calculated from day 7 – 118
8 Calculated from day 0 – 360

Conclusion:

2,4-D 2-EHE and 2,4-D were both much more persistent in foliage and leaf litter than soil with half-lives of 3-7.5 weeks. Leaf litter was shown to be the medium of highest persistence for all analytes measured, and 2,4-DCP and 2,4-DCA had half-lives of around 10 and 12 weeks respectively.

Pasture

Two field studies were provided for application of 2,4-D 2-EHE to pasture applied as Esteron 99C with the aim to determine the rate of dissipation of parent and metabolites. Both trials used a treated plot and an untreated control plot (UTC). They are summarised together.

Test Material:	**2,4-D 2-Ethylhexyl Ester**	**2,4-D 2-Ethylhexyl Ester**
Report:	Barney, 1995a.	Hatfield, 1995a
Guidelines:	US-EPA Subdivision N; 164-1	US-EPA Subdivision N; 164-1
GLP:	Yes	

Test System

Location:	Pattison, Texas.	Tulare County, California.
Plot sampling:	5 sampling areas in the treated plot; 3 in the UTC.	Three sampling areas in the treated plot; 1 in the UTC. Upwind buffer of around 60 m.

Soil texture in the top 15 cm.

Location	Texture	% Sand	% Silt	% Clay	% OM	CEC (meq/100 g)	pH	% MC
Texas	Silt loam	38.8	50.0	11.2	1.57	3.26	6.0	11.41
California	Sandy loam	55	35	10	3.9	15.1	7.9	21

The bulk density of the Texas soil was 1.57 g/cm^3 while that for the Californian soil was 0.96 g/cm^3.

Experimental treatments: Two applications were made, the first when weeds were immature and the second between 21 and 30 days after the first application. Equipment used was standard commercial ground application equipment sprayed in a volume of 10 gallons/acre (around 95 L/ha). The boom height was 18-19 inches (46-48 cm) above the crop. The target application rate was 2.25 kg ae/ha (Barney, 1995a) and 2.5 kg ae/ha (Hatfield, 1995a) at both application points in their respective studies.

An aliquot of spray mixture was taken for analysis prior to and after each spraying. In addition, verification of the application rate was carried out using application monitors. To determine the stability of the analytes throughout the chain of custody, field spikes of soil cores were prepared. Samples of water were collected at each irrigation event, except for two occasions in Hatfield, 1995a.

Sampling: Sampling was undertaken according to the following regime:

Texas: soil samples were collected from the untreated and treated plots at –1, 0, 1, 3, 7 and 14 DAT for the first application, and –1, 0, 1, 3, 7, 14, 30, 60, 90, 120 and 180 DAT for the second application. The second application was 26 days after the first.

California: soil samples were collected from the untreated and treated plots at –1, 0, 1, 3 and 7 DAT for the first application, and -1, 0, 1, 3, 7, 15, 30, 64, 94, 120 and 175 DAT for the second application. The second application was 30 days after the first.

Soil cores were taken to a depth of 48 inches (122 cm). The top 24" (61 cm) were cut into 15 cm segments and composited by depth increment. The 61-122 cm segments were stored for possible future analysis.

Analysis	Soil residue data were generated for 2,4-D 2-EHE, 2,4-D, 2,4-DCP and 2,4-DCA following extraction from soil by a combination of three solvent systems and sonication. The combined extracts were diluted with water and concentrated on a solid phase extraction cartridge. The analytes were eluted from the cartridge using two specific solvent systems yielding two fractions. These were combined into a single solution for chromatographic analysis.

Results and Discussion

Application verification:	Recoveries achieved on analysis of the application monitors at the Californian trial were 75% (application 1) and 67% (application 2). These recoveries were the mean of 9 separate monitors. Recoveries achieved on analysis of the application monitors at the Texas trial were 93% (application 1) and 87.6% (application 2). These recoveries were the mean of 15 separate monitors.
Findings:	Table A2.8 summarises the residue formation and decline of parent compound and metabolites in the Texas trial while Table A2.9 does the same for the Californian trial.

Table A2.8: Residue Formation and Decline in the Texas Trial (ppm).

Time	Cumulative	2,4-D 2-EHE	2,4-D	2,4-DCP	2,4-DCA
0 DA1A	0	0.289	0.225	-	-
1 DA1A	1	0.137	0.323	-	-
3 DA1A	3	-	0.230	-	-
7 DA1A	7	0.018	0.229	0.01	0.008
14 DA1A	14	-	0.071	0.009	0.008
-1 DA2A	30	-	0.048	0.005	-
0 DA2A	31	0.156	0.181	-	-
1 DA2A	32	0.133	0.253	-	-
3 DA2A	34	0.028	0.136	-	-
7 DA2A	38	0.119	0.006	-	-
14 DA2A	45	0.015	0.070	0.013	-
30 DA2A	61	-	0.077	-	-
60 DA2A	91	-	0.041	-	-
90 DA2A	121	-	0.017	-	-
120 DA2A	151	-	-	-	-
180 DA2A	211	-	-	-	-

Residues of 2,4-D 2-EHE remained in the upper 15 cm soil layer. 2,4-D was detected in one replicate at one interval (1DA1A) in the 15-30 cm layer. Also, at 14 DA1A, 2,4-D was detected throughout the 60 cm soil layer. The authors consider this is most likely to be due to sample contamination and not leaching as there were no other detections below the top 15 cm throughout the study.

Residues of the metabolites 2,3-DCP and 2,4-DCA were detected only in the top 15 cm layer. DEH has calculated the half-lives of 2,4-D residues following the first and second applications based on the equation $T1/2 = -\ln(2)/m$. For the first application, $m = -0.0628$, $r^2 = 0.87$ and the calculated half-life was 11 days which is in good agreement with the value calculated by the authors of 10.6 days. Following the second application (and ignoring the 7 DA2A outlier), $m = -0.0254$, $r^2 = 0.9035$ and the calculated half-life was 27.3 days which agrees with that calculated by the authors. 2,4-D 2-EHE was shown to degrade rapidly with calculated half-lives of 1.8 to 4.7 days reported by the authors.

Table A2.9: Residue Formation and Decline in the Californian Trial (ppm).

Time	Cumulative	2,4-D 2-EHE	2,4-D	2,4-DCP	2,4-DCA
0 DA1A	0	0.426	0.537	-	-
1 DA1A	1	0.084	0.679	0.012	-
3 DA1A	3	0.038	0.775	0.016	-
7 DA1A	7	0.009	0.489	0.010	-
-1 DA2A	25	-	0.012	-	-
0 DA2A	26	0.077	0.276	0.007	-
1 DA2A	27	0.018	0.278	-	-
3 DA2A	29	-	0.220	-	-
7 DA2A	33	-	0.100	-	-
15 DA2A	41	-	0.049	-	-
30 DA2A	56	-	0.050	-	-
64 DA2A	90	-	0.008	-	-
94 DA2A	120	-	-	-	-
120 DA2A	146	-	-	-	-

Residues of 2,4-D 2-EHE and 2,4-D were higher in soil after the first application when the pasture was mowed to a height of 5 cm compared to the second application when the pasture was at a height of 20-25 cm.

2,4-D 2-EHE, 2,4-DCP and 2,4-DCA were not detected below the 15 cm soil horizon at any sampling time in any replicate throughout the study. 2,4-D was found in a single replicate in the 15-30 cm soil layer at a concentration of 0.018 ppm the first day after the first application and again in a single replicate in this soil layer at a concentration of 0.011 ppm on the first day after the second application. No other detections of 2,4-D were found at any level below the top 15 cm layer.

Half-lives were calculated for dissipation curves using the slope derived from the regression model in the equation T1/2 = -ln(2)/m. 2,4-D 2-EHE exhibited a half-life of 1.4 days (r^2 = 0.9017) after the first application and 0.5 days (r^2 = 1.0) after the second application. 2,4-D exhibited a half-life of 4.2 days (r^2 = 0.942) after the first application and 13.1 days (r^2 = 0.913) after the second. As with the Californian study, interception was much greater with the second application (pasture height 20-25 cm) compared to that at the first application (5 cm), hence residues were higher following the first application.

Conclusions:

These studies demonstrate that when pasture is treated with two consecutive broadcast applications of the 2-EHE of 2,4-D up to a total rate of almost 5 kg ae/ha, 2,4-D 2-EHE, 2,4-D, 2,4-DCP and 2,4-DCA are relatively immobile in sandy loam soil and silt loam soil. Dissipation is fast for 2,4-D 2-EHE with half-lives less than 5 days and slower for 2,4-D with half-lives up to 4 weeks.

Wheat

Two field studies were provided for application of 2,4-D 2-EHE to wheat applied as Esteron 99C with the aim to determine the rate of dissipation of parent and metabolites. Both trials used a treated plot and an untreated control plot (UTC). They are summarised together.

Test Material:	**2,4-D 2-Ethylhexyl Ester**	**2,4-D 2-Ethylhexyl Ester**
Report:	Barney, 1995b.	Silvoy, 1995a
Guidelines:	US-EPA Subdivision N; 164-1	US-EPA Subdivision N; 164-1
GLP:	Yes	Yes
Test System		
Location:	Rowland, North Carolina.	Eaton, Colorado.
Plot sampling and irrigation:	5 sampling areas in the treated plot; 3 in the UTC. The treated plots were located between 80 and 110 m downslope from the UTC. Irrigation was applied as needed to the test areas to assure 110% or more of each monthly precipitation average found during the previous 30 year period.	

Soil texture in the top 15 cm.

Location	Texture	% Sand	% Silt	% Clay	% OM	CEC (meq/100 g)	pH	% MC
Nth Carolina	Sand	92.8	4.0	3.2	0.88	1.55	5.4	3.38
Colorado	Sandy clay loam	51.2	23.6	25.2	1.71	13.41	7.9	20.23

The bulk density of the North Carolina soil was 1.62 g/cm^3 while that for the Colorado soil was 1.44 g/cm^3

Experimental treatments:

Two applications were made. Both protocols state that the first should occur when the wheat would be fully tillered (10-20 cm high) but before forming joints in the stem. The second should be when the wheat is in the dough stage, around 60 days after the first application. In the North Carolina study, the first application was made when the wheat was at the booting crop stage (crop height 40-50 cm) and the second when the wheat was at the dough/kernel stage (height around 46 cm). This deviation is noted. There would be greater interception at the first application than expected if the protocol was followed. However, a bare ground study on a wheat area has been performed and the results of this study will be considered separately.

Equipment used was standard commercial ground application equipment sprayed in a volume of 10-11.5 gallons/acre (around 95 – 107 L/ha). The boom height was 19-24 inches (48-61 cm) above the crop. The target application rate was 1.4 kg ae/ha for both applications in both studies.

An aliquot of spray mixture was taken for analysis prior to and after each spraying. In addition, verification of the application rate was carried out using application monitors. To determine the stability of the analytes throughout the chain of custody, field spikes of soil cores were prepared. Samples of water were collected at each irrigation event, for analysis.

Sampling

Sampling was undertaken according to the following regime:

North Carolina: soil samples were collected from the untreated and treated plots at –1, 0, 1, 3, 6, 14, 30 and 60 (four days prior to the second application) DAT for the first application, and 0, 1, 2, 8, 15, 30, 62, 91, 120 and 181 DAT for the second application.

Colorado: soil samples were collected from the untreated and treated plots at –1, 0, 1, 3, 7, 15, 30 and 59 (one day prior to the second application) DAT for the first application, and –11, 0, 1, 3, 7, 15, 30, 60, 90, 120 and 180 DAT for the second application.

Soil cores were taken to a depth of 48 inches (122 cm) except for the days of applications when only the top 15 cm was sampled. The top 24" (61 cm) were cut into 15 cm segments and composited by depth increment. The 61-122 cm segments were stored for possible future analysis.

Analysis

Soil residue data were generated for 2,4-D 2-EHE, 2,4-D, 2,4-DCP and 2,4-DCA following extraction from soil by a combination of three solvent systems and sonication. The combined extracts were diluted with water and concentrated on a solid phase extraction cartridge. The analytes were eluted from the cartridge using two specific solvent systems yielding two fractions. These were combined into a single solution for chromatographic analysis.

Results and Discussion

Application verification:

Recoveries achieved on analysis of the application monitors at the North Carolina trial were 94% (application 1) and 104% (application 2).

Recoveries achieved on analysis of the application monitors at the Colorado trial were 95% (application 1) and 117% (application 2).

These recoveries were the mean of 15 separate monitors corrected for overall recovery.

Findings:

Table A2.10: Residue Formation and Decline in the North Carolina Trial (ppm).

Time	Cumulative	2,4-D 2-EHE	2,4-D	2,4-DCP	2,4-DCA
0 DA1A	0	0.016	0.265	0.004	-
1 DA1A	1	0.043	0.164	0.016	-
3 DA1A	3	0.016	0.146	0.017	-
6 DA1A	6	0.017	0.145	0.021	-
14 DA1A	14	-	0.06	0.017	-
30 DA1A	30	-	0.024	-	-
60 DA2A	60	-	-	-	-
0 DA2A	64	0.037	0.096	-	-
1 DA2A	65	0.006	0.119	-	-
2 DA2A	66	0.017	0.148	-	-
8 DA2A	72	-	0.058	-	-
15 DA2A	79	-	-	-	-
30 DA2A	94	-	0.007	-	-
62 DA2A	126	-	-	-	-
91 DA2A	155	-	-	-	-

No residues of any analyte were found at any sampling time below the top 15 cm soil layer. Irrigation water was tested for the presence of the three analytes found in soil as well as 4-CPA and 4-CP. No residues of 2,4-D or its potential degradates were found in any sample of irrigation water from the test site.

Least squares linear regression was performed with both the 2,4-D 2-EHE and 2,4-D, and the combined values of these analytes on an acid equivalent basis using the LINEST function in Microsoft Excel, version 5, spreadsheet software. Half-lives were calculated for dissipation curves using the slope derived from the regression model in the equation T1/2 = -ln(2)/m.

2,4-D 2-EHE exhibited a half-life of 4.0 days (r^2 = 0.62 based on 1 DAT to 6 DAT residues) after the first application and 1.8 days (r^2 = 0.19) after the second application. 2,4-D exhibited a half-life of 9.3 days (r^2 = 0.96) after the first application and 6.2 days (r^2 = 0.75) after the second application. Combining residues of these two analytes on an acid equivalent basis, the half-life after the first application was 8.9 days (r^2 = 0.97) and after the second application was 6.0 days (r^2 = 0.76).

Table A2.11: Residue Formation and Decline in the Colorado Trial (ppm).

Time	Cumulative	2,4-D 2-EHE	2,4-D	2,4-DCP	2,4-DCA
0 DA1A	0	0.047	0.157	-	-
1 DA1A	1	0.070	0.252	-	-
4 DA1A	4	0.016	0.272	0.012	-
7 DA1A	7	0.007	0.259	-	-
15 DA1A	15	0.003	0.063	-	0.007
30 DA1A	30	-	-	-	-
-4 DA2A	56	-	-	-	-
0 DA2A	60	0.074	0.166	-	-
1 DA2A	61	0.093	0.246	-	-
4 DA2A	64	0.029	0.230	-	-
7 DA2A	67	0.008	0.038	-	-
14 DA2A	74	-	0.008	-	-
31 DA2A	91	-	-	-	-
60 DA2A	120	-	-	-	-

No residues of any analyte were found at any sampling time below the top 15 cm soil layer. Irrigation water was tested for the presence of the three analytes found in soil as well as 4-CPA and 4-CP. No residues of 2,4-D or its potential degradates were found in any sample of irrigation water from the test site.

Least squares linear regression was performed with the mean concentrations of 2,4-D 2-EHE and 2,4-D across time after applications using the LINEST function in Microsoft Excel, version 5, spreadsheet software. Half-lives were calculated for dissipation curves using the slope derived from the regression model in the equation T1/2 = -ln(2)/m.

The authors calculate rapid degradation after the first application with 2,4-D 2-EHE having a half-life of 2.2 days (r^2 = 0.91) and 2,4-D a half-life of 4.8 days (r^2 = 0.96). Dissipation after the second application was faster still with respective half-lives being 2.1 days and 2.5 days.

DEH has recalculated these half-lives based on the slope derived from the regression model in the equation T1/2 = -ln(2)/m. Following the first application, 2,4-D 2-EHE has a calculated half-life of 3.4 days (r^2 = 0.89) and 2,4-D a half-life of 6.5 days (r^2= 0.82 based on day 1-15 data). The respective half-lives following the second application were calculated to be 2.0 days (r^2 = 0.95) and 2.8 days (r^2 = 0.89).

Conclusions:

These studies demonstrate that when wheat is treated with two consecutive broadcast applications of the 2-EHE of 2,4-D up to a total rate of 2.5 kg ae/ha, 2,4-D 2-EHE, 2,4-D, are relatively immobile in sandy loam soil and silt loam soil. Dissipation is fast for both analytes with half-lives less than 10 days at both field sites.

Turf

Two field studies were provided for application of 2,4-D 2-EHE to turf applied as Esteron 99C with the aim to determine the rate of dissipation of parent and metabolites. Both trials also considered residues and decline in grass clippings while the Californian study further considered residues and decline in thatch. Both trials used a treated plot and an untreated control plot (UTC). They are summarised together.

Test Material:	**2,4-D 2-Ethylhexyl Ester**	**2,4-D 2-Ethylhexyl Ester**
Report:	Barney, 1995c.	Hatfield, 1995b
Guidelines:	US-EPA Subdivision N; 164-1	US-EPA Subdivision N; 164-1
GLP:	Yes	Yes
Test System		
Location:	Rowland, North Carolina.	Porterville, California.
Plot sampling and irrigation:	5 sampling areas in the treated plot; 3 in the UTC. The treated plots were located between 41 and 200 m downslope from the UTC. Irrigation was applied as needed to the test areas to assure 110% (North Carolina) and 120% (California) or more of each monthly precipitation average found during the previous 30 year period.	

Soil texture in the top 15 cm:

Location	Texture	% Sand	% Silt	% Clay	% OM	CEC (meq/100 g)	pH	% MC
Nth Carolina	Sand	92.8	4.0	3.2	0.93	1.69	6.9	3.0
California	Loamy sand	84	12	4	0.7	4.9	6.3	6.3

The bulk density of the North Carolina soil was 1.61 g/cm^3 while that for the Californian soil was 1.29 g/cm^3

Experimental treatments:

Two applications were made. Both protocols state that the first should occur when weeds are immature (early spring) with the second to occur 21 days after the first.

Equipment used was standard commercial ground application equipment sprayed in a volume of 30 gallons/acre (around 280 L/ha). The boom height was 18-20 inches (46-51 cm) above the soil. The target application rate was 2.24 kg a e/ha in the North Carolina trial and 2.46 kg a e/ha in the Californian trial.

An aliquot of spray mixture was taken for analysis prior to and after each spraying. In addition, verification of the application rate was carried out using application monitors. To determine the stability of the analytes throughout the chain of custody, field spikes of soil cores were prepared. Samples of water were collected at each irrigation event, for analysis.

Sampling

Soil sampling was undertaken according to the following regime:

North Carolina: soil samples were collected from the untreated and treated plots at –1, 0, 1, 3, 6, 12 and 20 (one day prior to the second application) DAT for the first application, and 0, 1, 2, 5, 14, 35, 62, 93 and 120 DAT for the second application.

California: soil samples were collected from the untreated and treated plots at –1, 0, 1, 3, 7, 14 and 20 (two days prior to the second application) DAT for the first application, and 0, 1, 3, 7, 14, 30, 60, 90 and 120 DAT for the second application. The report claims soil sampling was meant to be done a day prior to the second application, however, the second application was changed from 27 April 1994 until 28 April 1994 due to weather conditions. Therefore, this took place 22 days after the first application.

Soil cores were taken to a depth of 48 inches (122 cm) except for the days of applications when only the top 15 cm was sampled. The top 24" (61 cm) were cut into 15 cm segments and composited by depth increment. The 61-122 cm segments were stored for possible future analysis.

North Carolina: **Grass** was sampled by hand cutting the grass obtained from the 5.7 cm diameter 0-15 cm soil cores. Samples were collected from the treated and UTC plots following the soil sampling regime above.

California: **Grass** clippings were obtained by mowing the plots with clippings wind-rowed near the plot centre and grab samples taken randomly. **Thatch** samples were obtained with the 0-15 cm soil layer. The sampling regime for both followed that for soil above.

Analysis

Soil residue data were generated for 2,4-D 2-EHE, 2,4-D, 2,4-DCP and 2,4-DCA following extraction from soil by a combination of three solvent systems and sonication. The combined extracts were diluted with water and concentrated on a solid phase extraction cartridge. The analytes were eluted from the cartridge using two specific solvent systems yielding two fractions. These were combined into a single solution for chromatographic analysis.

To analyse residues in grass, a grass sample was shaken for one hour in the presence of basic methanol, filtered then brought to a known volume. An aliquot of the methanol was evaporated, swamped with acidified water and passed through an SPE column. The 2,4-D is eluted from the column with two solvent systems, concentrated and derivatised to its methyl ester. The reactants were swamped with water and the methyl ester partitioned to a known volume of hexane from which an aliquot was diluted and analysed using gas chromatography/mass selective detection (GC/MSD).

Results and Discussion

Application verification:

Recoveries (15 monitoring pads per application) achieved on analysis of the application monitors at the North Carolina trial were 129% (application 1) and 112% (application 2) of the proposed study rates.

Recoveries (9 monitoring pads per application) achieved on analysis of the application monitors at the Californian trial were around 88% (application 1) and 102% (application 2) of the proposed study rates.

Findings: **SOIL**

Table A2.12: Residue Formation and Decline in Soil in the North Carolina Trial (ppm).

Time	Cumulative	2,4-D 2-EHE	2,4-D	2,4-DCP	2,4-DCA
0 DA1A	0	0.031	0.376	-	-
1 DA1A	1	0.004	0.425	0.004	-
3 DA1A	3	-	0.225	0.011	-
6 DA1A	6	-	0.088	0.007	0.018
12 DA1A	12	-	0.039	-	0.009
-1 DA2A	20	-	0.022	-	-
0 DA2A	21	0.017	0.615	-	-
1 DA2A	22	-	0.389	-	-
2 DA2A	23	-	0.368	0.004	0.003
5 DA2A	26	-	0.038	-	-
14 DA2A	35	-	0.008	-	-
35 DA2A	56	-	-	-	-
62 DA2A	83	-	-	-	-

Soil residues of 2,4-D 2-EHE and the 2,4-DCP and 2,4-DCA degradates were found only in the top 15 cm of soil. Residues of 2,4-D acid were mainly found in this top layer but were also found in the 15-30 cm layer at 1, 3 and 6 days after the first application at mean concentrations of 0.012, 0.028, and 0.018 ppm respectively. After the second application, 2,4-D was found in the 15-30 cm layer at 1 and 5 DAT at levels of 0.01 ppm on both occasions. One anomalous detection was found in the 30-45 cm layer at 12 days after the first treatment but not in the layer above this at this time. No residues were found in any soil samples taken 35 days or more after the second application.

Least squares linear regression was performed with both the 2,4-D 2-EHE and 2,4-D, and the combined values of these analytes on an acid equivalent basis using the LINEST function in Microsoft Excel, version 5, spreadsheet software. Half-lives were calculated for dissipation curves using the slope derived from the regression model in the equation T1/2 = -ln(2)/m.

2,4-D 2-EHE exhibited a half-life of 0.34 days (r^2 = 1.0) after the first application and <1 day after the second (no calculation was possible as there was only one data point). 2,4-D exhibited half-lives of 4.4 days (r^2 = 0.94) after the first application and 2.2 days (r^2 = 0.94) after the second. The total of these two analytes on an acid-equivalent basis resulted in half-lives of 4.4 days (r^2 = 0.94) and 2.2 days (r^2 = 0.94) after the first and second applications respectively.

Table A2.13: Residue Formation and Decline in Soil in the Californian Trial (ppm).

Time	Cumulative	2,4-D 2-EHE	2,4-D	2,4-DCP	2,4-DCA
0 DA1A	0	0.021	0.058	-	-
1 DA1A	1	0.034	0.055	-	-
3 DA1A	3	0.009	0.061	-	-
7 DA1A	7	-	0.022	-	-
14 DA1A	14	-	0.04	-	-
-2 DA2A	20	-	0.004	-	-
0 DA2A	22	0.022	0.130	-	-
1 DA2A	23	0.036	0.108	-	-
3 DA2A	25	-	0.042	-	-
7 DA2A	29	-	0.091	-	-
14 DA2A	36	-	0.019	-	-
30 DA2A	52	-	0.014	-	-
60 DA2A	82	-	-	-	-

Residues of 2,4-D 2-EHE were retained in the top 15 cm soil at all sampling times where it was detected. 2,4-DCP and 2,4-DCA were not detected at any time with the exception of 0.004 ppm 2,4-DCP being found in the 30-45 cm layer 3 days after the second application. Residues of 2,4-D were detected in most sampling intervals until 30 days after the second application. 14 days after the first application and 3 days after the second, 2,4-D was found down to the 30-45 cm layer at concentrations of 0.019 ppm and 0.008 ppm respectively. 7 days after the second application, 2,4-D was found in the three soil layers from 60-100 cm, but not in any higher layers except the top 15 cm. The authors believe these detections are due to sample contamination in grass.

Linear regression analysis was performed to determine half-lives. The study reports a half-life of 2.1 days in soil for 2,4-D 2-EHE. 2,4-D half-lives are reported to be 7.9 days (r^2 = 0.84) following the first application and 9.7 days (r^2 = 0.75) after the second.

Findings: **Grass and Thatch**

Table A2.14: 2,4-D Mean Residues (ppm) in Grass and Thatch.

North Carolina Trial		Californian Trial		
Time	Grass	Time	Grass	Thatch
0 DA1A		0 DA1A	153.2	6.43
1 DA1A	29.3	1 DA1A	135.97	4.38
3 DA1A	22.0	3 DA1A	86.40	4.33
6 DA1A	16.6	7 DA1A	59.50	5.04
12 DA1A	7.9	14 DA1A	30.57	1.12
-1 DA2A	7.4	-2 DA2A	11.97	1.87
0 DA2A	43.9	0 DA2A	184.33	2.47
1 DA2A	67.6	1 DA2A	202.00	3.35
2 DA2A	42.3	3 DA2A	160.33	5.03
5 DA2A	19.7	7 DA2A	137.33	2.76
14 DA2A	19.7	14 DA2A	54.93	1.49
35 DA2A	2.3	30 DA2A	28.33	0.00
62 DA2A	0.0	60 DA2A	2.13	0.00

1) Samples for the 0 DA1A interval were not analysed because samples were inadvertently not shipped to the testing facility.

Dissipation curves have been calculated by DEH using the slope derived from the regression model in the equation T1/2 = -ln(2)/m.

Based on mean residues, the half-life of 2,4-D in grass in the North Carolina trial was calculated to be 9.3 days ($r^2 = 0.88$) after the first application and 8.0 days ($r^2 = 0.93$) after the second application.

Residues found in grass in the Californian study are significantly higher than those found in the North Carolina trial. One possible reason for this is the height of turf at application. It is known in the North Carolina study, the turf was around 7.5 cm high at the time of the first application and around 5 cm high at the second (hence the higher residues in this trial following the second application). The height of the turf in the Californian study is not specified. Based on mean residues, the half-life of 2,4-D in grass in the Californian trial was calculated to be 5.7 days ($r^2 = 0.99$) after the first application and 9.3 days ($r^2 = 0.99$) after the second application.

Thatch residues in the Californian study were significantly less than those found on grass. However, persistence was slightly longer. Based on mean residues, the half-life of 2,4-D in thatch was calculated to be 9.3 days ($r^2 = 0.70$) after the first application and 12.9 days ($r^2 = 0.47$) after the second application. A slightly better correlation coefficient can be obtained by ignoring the apparent outlier at 3 DA2A where a half-life of 15.8 days is predicted with $r^2 = 0.68$.

Conclusions:

These studies demonstrate that when turf is treated with two consecutive broadcast applications of the 2-EHE of 2,4-D up to a total rate of almost 5 kg ae/ha (combined rate), 2,4-D 2-EHE, 2,4-D, are relatively immobile in sandy loam soil and silt loam soil. Dissipation is fast for both analytes with half-lives less than 10 days at both field sites. 2,4-D 2-EHE dissipated faster than 2,4-D.

2,4-D dissipated from grass at a rate similar to that in soil with half-lives less than 10 days for both trial sites. Thatch residues of 2,4-D were analysed for the Californian trial only and were found at significantly less levels than on grass. However, dissipation was slower, particularly after the second application where a half-life of around 16 days was predicted.

Granules

Two field studies were provided for application of 2,4-D 2-EHE in the granular form, Riverdale Weedestroy Weed and Feed (1.0% ae granular). Both trials were undertaken in Ohio, one on bare ground, the other on turf. Both used a treated plot and an untreated control plot (UTC). They are summarised together.

Test Material:	**2,4-D 2-Ethylhexyl Ester**	**2,4-D 2-Ethylhexyl Ester**
Report:	Hatfield, 1995c.	Hatfield, 1995d
Guidelines:	US-EPA Subdivision N; 164-1	US-EPA Subdivision N; 164-1
GLP:	Yes	Yes
Test System	Bare Ground	Turf
Location:	Fayette County, Ohio.	Fayette County, Ohio.
Plot sampling and irrigation:	5 sampling areas in the treated plot; 3 in the UTC. The treated plots were located about 43 m downslope from the UTC. Protocols state that irrigation was to be applied as needed to the test areas to assure at least 120% of each monthly precipitation average found during the previous 10 year period.	

Soil texture in the top 15 cm:

Situation	Texture	% Sand	% Silt	% Clay	% OM	CEC (meq/100 g)	pH	% MC
Bare Ground	Silt loam	33	52	15	4.0	13.9	6.5	31.9
Turf	Silt loam	27	53	20	5.0	24.5	6.5	32.8

The bulk density of both soils was between 1.03 and 1.09 g/cm^3

Experimental treatments: For both tests, the treated plots received two applications with a target rate of 2.46 kg ae/ha each time. The first application was in early spring and the second 21 days later.

Equipment used was standard commercial ground application equipment using a broadcast drop spreader. The boom height was 11 inches (28 cm) above either the turf height or the ground surface. A sample of test substance was taken for analysis prior to and after each spraying. To verify application rates, time and direction of applicator passes were recorded. In addition, test substances remaining after application were weighed and subtracted from the starting weight of the product. To determine the stability of the analytes throughout the chain of custody, field spikes of soil cores were prepared. Samples of water were collected at each irrigation event, for analysis.

Sampling	**Soil sampling** was undertaken according to the following regime for both trials: Soil samples were collected from the untreated and treated plots at –1, 0, 1, 3, 7, 14 and 20 (one day prior to the second application) DAT for the first application, and 0, 1, 3, 7, 14, 30, 60, 90 and 120 DAT for the second application. Soil cores were taken to a depth of 48 inches (122 cm) except for the days of applications when only the top 15 cm was sampled. The top 24" (61 cm) were cut into 15 cm segments and composited by depth increment. The 61-122 cm segments were stored for possible future analysis. In the turf test, **grass** clippings were obtained by mowing the plots with clippings wind-rowed near the plot centre and grab samples taken randomly. Approximately 440 g of clippings were taken from each sub-plot. **Thatch** samples were obtained with the 0-15 cm soil layer. The sampling regime for both followed that for soil above.
Analysis	Soil residue data were generated for 2,4-D 2-EHE, 2,4-D, 2,4-DCP and 2,4-DCA following extraction from soil by a combination of three solvent systems and sonication. The combined extracts were diluted with water and concentrated on a solid phase extraction (SPE) cartridge. The analytes were eluted from the cartridge using two specific solvent systems yielding two fractions. These were combined into a single solution for chromatographic analysis. Grass and thatch samples were analysed for 2,4-D and 2,4-D 2-EHE by extraction in basic methanol (converting 2,4-D 2-EHE to 2,4-D). The extract was filtered, an aliquot reduced, swamped with filtered acidified water and passed through an SPE column. The eluant was concentrated, methylated and swamped with deionised water then partitioned to hexane. The hexane extract was diluted five-fold with hexane and quantitated using gas chromatography/mass selective detection (GC/MSD).

Results and Discussion

Application verification:	In the bare ground study, the rate applied for applications 1 and 2 was calculated to be 95% and 86% respectively. The lower rate for the second application was considered due to the inconsistent quality of the fertiliser material that the chemical was impregnated on. Calculated application rates for the turf trial were 98 and 96% for applications 1 and 2 respectively.
Findings:	**SOIL**

Table A2.15: Residue Formation and Decline in Soil in the Bare Ground Trial (ppm).

Time	Cumulative	2,4-D 2-EHE	2,4-D	2,4-DCP	2,4-DCA
0 DA1A	0	0.954	0.148		
1 DA1A	1	1.361	0.245		
3 DA1A	3	0.443	0.270		
7 DA1A	7	0.352	0.367	0.013	
14 DA1A	14	0.353	0.665	0.023	0.005
-1 DA2A	20	0.129	0.586	0.015	
0 DA2A	21	0.996	0.947	0.016	
1 DA2A	22	0.531	0.704	0.020	
3 DA2A	24	0.501	0.940	0.027	0.004
7 DA2A	28	0.373	1.015	0.030	
14 DA2A	35	0.293	1.301	0.029	
30 DA2A	51	0.024	0.029	0.003	0.010
60 DA2A	81		0.014	0.011	
90 DA2A	111		0.004	0.007	

Residues were mainly confined to the top 15 cm of soil. However, on 5 sampling occasions, residues of both 2,4-D 2-EHE and 2,4-D were found in the 15-30 cm layer, at 3, 7 and 14 days after the first application and 3 and 14 days after the second. Maximum residues of 2,4-D 2-EHE found in this layer were 0.013 ppm at 3 days after the 1st application (about 1.3% of the maximum residues of this chemical found in the top soil layer). Maximum residues of 2,4-D were 0.051 ppm in the 15-30 cm layer on 14 days after the second application (about 3.9% of the maximum residues found in the top layer).

Least squares linear regression was performed with both the 2,4-D 2-EHE and 2,4-D, calculated for dissipation curves using the slope derived from the regression model in the equation $T1/2 = -\ln(2)/m$.

2,4-D 2-EHE exhibited a half-life of 7.4 days ($r^2 = 0.81$) after the first application and 6.3 days ($r^2 = 0.94$) after the second. After the first application, concentrations of 2,4-D continued to increase due to conversion of the test substance into its metabolites. This results in a positive slope and negative correlation to dissipation. The half-life is therefore only calculated from the second application residue data. It appears that residues of 2,4-D will continue to climb until around 14 days after application with granules. The report calculates a half-life of 6.6 days ($r^2 = 0.70$) following the second application. DEH has re-calculated these data and determines a half-life of 10.3 days ($r^2 = 0.885$).

The report does not calculate a dissipation half-life for 2,4-DCP. The data indicate a pattern of decline can be observed. Using residue data following the second application, a half-life of 48 days ($r^2 = 0.34$) is calculated. If the apparent outlier of 0.003 ppm at 30 DA2A is removed, the r^2 is significantly improved (0.75) and the calculated half-life is 51.7 days.

Table A2.16: Residue Formation and Decline in Soil in the Turf Trial (ppm).

Time	Cumulative	2,4-D 2-EHE	2,4-D	2,4-DCP	2,4-DCA
0 DA1A	0	0.085	0.055		
1 DA1A	1	0.171	0.084		
3 DA1A	3	0.280	0.098		
7 DA1A	7	0.129	0.143	0.004	0.005
14 DA1A	14	0.076	0.104		0.006
-1 DA2A	20	0.059	0.129	0.004	0.015
0 DA2A	21	0.084	0.123		
1 DA2A	22	0.688	0.280		0.010
3 DA2A	24	0.098	0.215	0.007	0.006
7 DA2A	28	0.076	0.194		0.004
14 DA2A	35	0.056	0.156		0.004
30 DA2A	51	0.030	0.046	0.003	0.050
60 DA2A	81		0.009		0.035
90 DA2A	111			0.004	0.016
120 DA2A	141				0.012

Generally, residues were retained in the top 15 cm. However, on the first day after Application 1, 2,4-D 2-EHE (0.012 ppm) and 2,4-D (0.004 ppm) were found in the 15-30 cm layer. 2,4-D 2-EHE was also found in this layer at 0.004 ppm 3 days after Application 1 and 2,4-D at 0.004 ppm 7 days after application 1.

There were two detections of 2,4-D 2-EHE in the 30-45 cm layer, one at 14 days after the first application (0.024 ppm) and one at 1 day after application 2 (0.001 ppm). 2,4-D was found in this layer on one occasion, at 0.054 ppm 14 days after application 1.

2,4-DCP and 2,4-DCA were found exclusively in the top 15 cm of soil with the exception of one detection of 2,4-DCA (0.002 ppm) in the 15-30 cm layer at 30 days after the second application.

Least squares linear regression was performed with both the 2,4-D 2-EHE and 2,4-D, calculated for dissipation curves using the slope derived from the regression model in the equation T1/2 = -ln(2)/m.

2,4-D 2-EHE exhibited a half-life of 14.3 days ($r^2 = 0.45$) after the first application and 10.9 days ($r^2 = 0.47$) after the second. The poor correlation co-efficient may be due to the increase in residues over the first few days as chemical is released from the granule to the soil. The peak of 2,4-D 2-EHE in the soil following the first application was 0.28 ppm 3 DA1A. If the half-life is modelled from this time point, it is calculated to be 7.9 days ($r^2 = 0.91$).

After the first application, concentrations of 2,4-D continued to increase due to conversion of the test substance into its metabolites. This results in a positive slope and negative correlation to dissipation. The half-life is therefore only calculated from the second application residue data. It appears that residues of 2,4-D will continue to climb until around 14-20 days after application with granules. The report calculates a half-life of 13 days ($r^2 = 0.93$) following the second application.

The report does not calculate a dissipation half-life for 2,4-DCA. The data indicate a pattern of decline can be observed. Peak residues of this analyte were found 30 DA2A (0.05 ppm). Using these residue data following the second application, a half-life of 41 days ($r^2 = 0.97$) is calculated.

Findings: **Grass and Thatch**

Table A2.17: 2,4-D Mean Residues (ppm) in Grass and Thatch from Turf Trial.

Time	Grass	Thatch
0 DA1A	13.91	6.93
1 DA1A	21.13	11.24
3 DA1A	28.60	14.81
7 DA1A	16.98	7.51
14 DA1A	13.97	7.97
-1 DA2A	15.10	15.34
0 DA2A	38.83	8.68
1 DA2A	52.00	20.13
3 DA2A	39.93	12.37
7 DA2A	37.70	12.90
14 DA2A	42.97	16.30
30 DA2A	3.04	0.0
60 DA2A	0.84	0.0

1) Samples for the 0 DA1A interval were not analysed because samples were inadvertently not shipped to the testing facility.

In grass clippings a half-life following the first application was calculated to be 44 days for 2,4-D. The correlation co-efficient was poor ($r^2 = 0.19$) and it appeared to take some days for residues to peak in the grass. Following the second application, residues appeared to have peaked after the first day and the half-life was calculated to be 9.6 days ($r^2 = 0.92$).

Half-lives in thatch could not be calculated following the first application. The residues did not appear to peak between the first and second applications, and a negative correlation was therefore observed. While residues appeared to remain fairly constant following the second application until 14DA2A, no residues were detected by 30DA2A. For the purposes of estimating a half-life, if it is assumed that ½ LOQ is available at 30DA2A (ie, 0.5 ppm), a half-life of 6.9 days ($r^2 = 0.71$) is calculated.

Conclusions:

Application of granules appears to result in much slower conversion of 2,4-D 2-EHE to the 2,4-D acid. Residues of 2,4-D appear to peak between 14-20 days after granular application and then dissipate with a half-life around 10 days. The parent 2,4-D 2-EHE dissipates with a half-life around 1-2 weeks. The 2,4-DCA metabolite gradually increases in concentration before declining with a half-life in the area of 41 days.

Residue decline in grass clippings was difficult to gauge after the first application due to a stage of increasing residues being observed. However, after the second application, good correlation between dissipation and time was found with a half-life of around 10 days. Residues did not appear to peak in thatch samples throughout the study and a half-life could not be determined. However, no residues were found in thatch at the 30 DA2A or then on, so the residues were shown to dissipate.

Bare Soil

Seven field studies were provided for application of 2,4-D 2-EHE applied as Esteron 99C with the aim to determine the rate of dissipation of parent and metabolites. All trials used a treated plot and an untreated control plot (UTC). They were all performed on sites in the Unites States of America following US EPA Subdivision N;

164-1 and were all undertaken according to GLP. The studies are summarised together.

Test Material:	**2,4-D 2-Ethylhexyl Ester**	
Report	**Author**	**Situation**
1 of 7	Hatfield, 1995e	Bare ground in California
2 of 7	Silvoy, 1995b	Bare ground in Colorado
3 of 7	Barney 1995d	Bare ground in North Carolina – Turf use pattern
4 of 7	Hatfield, 1995f	Bare ground in North Dakota – Wheat use pattern
5 of 7	Barney, 1995e	Bare ground in North Carolina – Wheat use pattern
6 of 7	Hatfield, 1995g	Bare ground in Nebraska – Corn use pattern
7 of 7	Hatfield, 1995h	Bare ground in Ohio – Corn use pattern

Plot sampling and irrigation: The treated plots were located downslope from the UTC. Protocols state that irrigation was to be applied as needed to the test areas to assure at least 110% of each monthly precipitation average found during the previous 10 year period. Where this didn't happen, deviations to the protocol were noted in the reports, and were usually the result of very wet conditions in preceding months resulting in ground being too moist.

Soil texture in the top 15 cm:

Report	Texture	% Sand	% Silt	% Clay	% OM	pH	CEC (meq/100 g)	% MC	Density (g/cm^3)
1 of 7	Loamy sand	78	18	4	1.2	6.7	7.2	7.9	1.28
2 of 7	Sandy clay loam	51.6	24.0	24.4	1.27	8.0	9.45	24.79	1.42
3 of 7	Sand	92.8	2.0	5.2	1.32	6.3	1.58	3.64	1.63
4 of 7	Sandy loam	62	24	14	3.1	5.9	21.0	22.4	1.12
5 of 7	Sand	88.8	6.0	5.2	1.11	6.6	1.94	3.77	1.63
6 of 7	Silt loam	18	61	21	2.9	5.7	16.5	24.1	1.13
7 of 7	Clay loam	23	47	30	2.0	7.0	17.3	24.9	1.14

Experimental treatments: In all tests, a sample of test substance was taken for analysis prior to and after each spraying. To verify application rates, time and direction of applicator passes were recorded. In addition, test substances remaining after application were weighed and subtracted from the starting weight of the product. Further, verification of the application rate was carried out using application monitors. To determine the stability of the analytes throughout the chain of custody, field spikes of soil cores were prepared. Samples of water were collected at each irrigation event, for analysis.

The following table provides a matrix of treatment rates

Table A2.18: Experimental Treatments for Bare Soil Field Dissipation Studies.

Report	Situation	No. applic.	Rate/app	Days apart	L/ha	Boom height
1 of 7	Bare	2	2.46 kg ae/ha	21	280	30 cm
2 of 7	Bare	2	1.4 kg ae/ha	60	93.5	Not stated
3 of 7	Turf	2	2.24 kg ae/ha	21	280	48-51 cm
4 of 7	Wheat	2	1.54 kg ae/ha	63	47	50-55 cm
5 of 7	Wheat	2	1.4 kg ae/ha	64	93.5	48-51 cm
6 of 7	Corn	4	2.46; 1.23; 0.62; 1.85	15; 30; 32	93.5	20
7 of 7	Corn	4	2.46; 1.23; 0.62; 1.85	19; 34; 89	93.5	30-46

Sampling **Soil sampling** was undertaken according to the following regimes:

Table A2.19: Soil sampling regimes.

Report	Situation	Application	Sampling regime
1 of 7	Bare	1	-1, 0, 1, 3, 7, 14 and 20 (1 day before second application)
		2	0, 1, 3, 7, 14, 30, 60, 90 and 120
2 of 7	Bare	1	-1, 0, 1, 3, 7, 15, 30, 59 (1 day before second application)
		2	0, 1, 3, 7, 14, 30, 60, 90, 120, 180, 360 and 540.
3 of 7	Turf	1	-1, 0, 1, 3, 6, 12 and 20 (1 day before second application)
		2	0, 1, 2, 5, 14, 35, 62, 93 and 120
4 of 7	Wheat	1	-1, 0, 1, 3, 7, 14, 30 and 62 (1 day before second appl^{n})
		2	0, 1, 3, 7, 14, 30, 60 and 90.
5 of 7	Wheat	1	-1, 0, 1, 3, 6, 14, 30 and 60 (4 days before second appl^{n})
		2	0, 1, 2, 8, 15, 30, 62, 91, 120 and 181.
6 of 7	Corn[1]	1	-1, 0, 1, 3, 7, 14 (one day before second application)
		2	0, 1, 3, 7, 14, 29 (one day before third application)
		3	0, 1, 3, 7, 14, 30, 60, 89, (one day before fourth appl^{n})
		4	0, 1, 3, 7, 14, 30, 60, 90 and 120.
7 of 7	Corn	1	-1, 0, 1, 3, 7, 18 (one day before second application)
		2	0, 1, 3, 7, 14, 33 (one day before third application)
		3	0, 1, 3, 7, 14, 30, 60, 88, (one day before fourth appl^{n})
		4	0, 1, 3, 7, 14, 30, 60, 90 and 120.

Analysis Soil residue data were generated for 2,4-D 2-EHE, 2,4-D, 2,4-DCP and 2,4-DCA following extraction from soil by a combination of three solvent systems and sonication. The combined extracts were diluted with water and concentrated on a solid phase extraction (SPE) cartridge. The analytes were eluted from the cartridge using two specific solvent systems yielding two fractions. These were combined into a single solution for chromatographic analysis.

Results and Discussion

Application rates were verified using monitoring pads. The summary of pad analysis for each application within each of the seven studies is summarised below in Table A2.20. Following this, Tables A2.21 through to A2.27 show the residue formation and decline in the top 15 cm of soil for the 7 studies, in the order they have been discussed above. Footnotes to each table are used to discuss movement of 2,4-D 2-EHE, 2,4-D and certain metabolites through the soil profile.

Application verification:

Table A2.20: Application Recoveries Based on Monitoring Pad Analysis:

Study	Application	Target rate (kg ae/ha)	Actual rate (kg ae/ha)	% Target
1 of 7	1	2.46	2.28	93
	2	2.46	2.14	87
2 of 7	1	1.4	1.26	90
	2	1.4	1.40	100
3 of 7	1	2.24	2.16	96
	2	2.24	2.81	125
4 of 7	1	1.54	1.12	73
	2	1.54	1.43	93
5 of 7	1	1.4	1.32	94
	2	1.4	1.50	107
6 of 7	1	2.46	1.79	73
	2	1.23	1.16	95
	3	0.62	0.52	84
	4	1.85	1.26	73
7 of 7	1	2.46	2.24	89
	2	1.23	1.15	90
	3	0.62	0.59	91
	4	1.85	1.68	88

Findings: **Residues**

Table A2.21: Residue Formation and Decline in Bare Ground Soil (ppm), Report 1 of 7.

Time	Cumulative	2,4-D 2-EHE	2,4-D	2,4-DCP	2,4-DCA
0 DA1A	0	0.200	0.410	-	-
1 DA1A	1	0.155[1]	0.458[2]	-	-
3 DA1A	3	0.187[3]	0.330[4]	-	-
7 DA1A	7	-	0.071	-	-
14 DA1A	14	0.005	0.061[5]	0.004	0.012
-1 DA2A	20	-	-[6]	-	-
0 DA2A	21	0.139	0.329	-	-
1 DA2A	22	0.086[7]	0.230[8]	-	0.005
3 DA2A	24	0.03[9]	0.282[10]	-	-
7 DA2A	28	0.021	0.266[11]	-	0.005[12]
14 DA2A	35	-	0.146[13]	-	0.005[14]
30 DA2A	51	-	-[15]	-	-[16]
60 DA2A	81	-	-	-	-
90 DA2A	111	-	-	-	-

1) Found in the 15-30 cm layer at 0.003 ppm
2) Found in the 15-30 cm layer at 0.008 ppm
3) Found in the 15-30 cm layer at 0.027 ppm
4) Found in the 15-30 cm layer at 0.024 ppm
5) 2,4-D was found through the whole profile down to 120 cm. However, around 78% of detections below the top 15 cm occurred in samples obtained from replicate "b" with the remainder from the "c" replicate in the 15-30 and 30-45 cm layers only. Therefore, the apparent concentrations at other depths are considered to most likely be a result of mechanical mixing.
6) No 2,4-D was found in the top 15 cm, however, it was detected at 0.052 ppm (15-30 cm); 0.165 ppm (30-45 cm) and 0.022 ppm (45-60 cm). The lower 60-120 cm was therefore sampled but no further detections were found.
7) 2,4-D 2-EHE was also found at 0.044 ppm (15-30 cm) and 0.009 ppm (30-45 cm).
8) 2,4-D was also found at various levels down to 60-75 cm where it was detected at 0.016 ppm.
9) 2,4-D 2-EHE was also found at 0.012 ppm (15-30 cm) and 0.004 ppm (45-60 cm).
10) 2,4-D was detected throughout the soil profile, down to 105-120 cm where it was found at 0.011 ppm.
11) 2,4-D was detected throughout the soil profile (no detections in 60-105 cm), down to 105-120 cm where it was found at 0.010 ppm.
12) 2,4-DCA was also found in the 30-45 cm at 0.004 ppm.
13) 2,4-D was detected throughout the soil profile. Six out of the eight detects below 15 cm came from the "c" replicate. Detects in the other replicates occurred in the 45-60 cm fraction.
14) 2,4-DCA was also found in the 15-30 cm layer at 0.004 ppm.
15) There were eight detections of 2,4-D from 30-120 cm. Five of these came from soils in replicate "a". One detect from replicate "c" occurred below the 60 cm depth. Subsequent intervals showed no quantifiable residues of any analytes.

Table A2.22: Residue Formation and Decline in Bare Ground Soil (ppm), Report 2 of 7.

Time	Cumulative	2,4-D 2-EHE	2,4-D	2,4-DCP	2,4-DCA
0 DA1A	0	0.115	0.218	-	-
1 DA1A	1	0.089	0.321	-	-
4 DA1A	4	0.031	0.243	-	-
7 DA1A	7	0.007	0.217	-	-
15 DA1A	15	-	0.128	0.013	-
30 DA1A	30	-	0.011	0.004	-
-4 DA2A	56	-	-	-	-
0 DA2A	60	0.164	0.403	0.021	-
1 DA2A	61	0.158	0.378[1]	0.020	-
3 DA2A	63	0.046	0.276	-	-
7 DA2A	67	0.004	0.156	-	-
14 DA2A	74	-	0.005	-	-
31 DA2A	91	-	-	-	-
60 DA2A	120	-	-	-	-

1) 2,4-D was found in one replicate in the 15-30 cm layer at 0.014 ppm (average of 3 replicates = 0.005 ppm)

Table A2.23: Residue Formation and Decline in Bare Soil (ppm) – Turf Use Pattern, Report 3 of 7.

Time	Cumulative	2,4-D 2-EHE	2,4-D	2,4-DCP	2,4-DCA
0 DA1A	0	0.271	0.264	-	-
1 DA1A	1	0.397[1]	0.613[2]	0.010	-
3 DA1A	3	0.028	0.351[3]	0.020	-
6 DA1A	6	0.004	0.072	0.012	0.012
12 DA1A	12	-	0.024	-	0.014
-1 DA2A	20	-	-	-	-
0 DA2A	21	0.247	0.436	-	-
1 DA2A	22	0.052	0.592[4]	0.003	-
2 DA2A	23	0.018	0.417[5]	0.008	-
5 DA2A	26	-	0.035	-	-
14 DA2A	35	-	0.003	-	-
35 DA2A	56	-	-	-	-
62 DA2A	83	-	-	-	-

1) 2,4-D 2-EHE found in the 15-30 cm soil layer at a mean concentration of 0.039 ppm.
2) 2,4-D found in the 15-30 cm soil layer at a mean concentration of 0.035 ppm.
3) 2,4-D found in the 15-30 cm soil layer at a mean concentration of 0.033 ppm.
4) 2,4-D found in the 15-30 cm soil layer at a mean concentration of 0.036 ppm.
5) 2,4-D found in the 15-30 cm soil layer at a mean concentration of 0.009 ppm.

3 of 7: Immediately after the 1st application, 0.6 inches (15 mm) of irrigation was applied to the plots and the following day, a further 7.5 mm of rain was recorded. Immediately after the 2nd application, 6.4 mm of irrigation was applied. The first rainfall after this application was 25.4 mm 10 days after application.

Table A2.24: Residue Formation and Decline, Bare Soil (ppm) – Wheat Use Pattern, 4 of 7.

Time	Cumulative	2,4-D 2-EHE	2,4-D	2,4-DCP	2,4-DCA
0 DA1A	0	0.347	0.160	-	-
1 DA1A	1	0.394	0.155	-	-
3 DA1A	3	0.447[1]	0.221[2]	-	-
7 DA1A	7	0.065	0.304	0.023	-
14 DA1A	14	0.016	0.024[3]	0.008	0.004
30 DA1A	30	0.005	0.008	-	-
-1 DA2A	62	-	-	-	-
0 DA2A	63	0.312	0.297	-	-
1 DA2A	64	0.254	0.316	-	-
3 DA2A	66	0.029	0.365	0.014	-
7 DA2A	70	0.018	0.173	0.014	-
14 DA2A	77	0.019	0.076	-	-
30 DA2A	93	-	0.009	-	-
60 DA2A	123	-	-	-	-

1) 2,4-D 2-EHE found in the 15-30 cm soil layer at a mean concentration of 0.009 ppm.
2) 2,4-D found in the 15-30 cm soil layer at a mean concentration of 0.004 ppm.
3) 2,4-D found in the 15-30 cm soil layer at a mean concentration of 0.005 ppm.

Table A2.25: Residue Formation and Decline, Bare Soil (ppm) – Wheat Use Pattern, 5 of 7.

Time	Cumulative	2,4-D 2-EHE	2,4-D	2,4-DCP	2,4-DCA
0 DA1A	0	0.028	0.261	-	-
1 DA1A	1	0.074	0.355	0.011	-
3 DA1A	3	0.054	0.384	0.022	-
6 DA1A	6	0.014	0.153	0.016	-
14 DA1A	14	0.025	0.107	0.015	-
30 DA1A	30	-	0.010	0.007	-
-4 DA2A	60	-	-	-	-
0 DA2A	64	0.065	0.146	-	-
1 DA2A	65	0.065	0.254	-	-
2 DA2A	66	0.05	0.314	0.007	-
8 DA2A	72	-	0.053	0.026	0.003
15 DA2A	79	-	0.009	-	-
30 DA2A	94	-	-	-	-
62 DA2A	125	-	-	-	-

5 of 7: No residues were found below 15 cm at any sampling time for any analyte. Immediately after the 1st application, 0.25 inches (6.4 mm) of irrigation was applied to the plots and the following day, a further 7.5 mm of rain was recorded. Immediately after the 2nd application, 6.4 mm of irrigation was applied. The first rainfall after this application was 27.9 mm 3 days after application.

Table A2.26: Residue Formation and Decline, Bare Soil (ppm) – Corn Use Pattern, 6 of 7.

Time	Cumulative	2,4-D 2-EHE	2,4-D	2,4-DCP	2,4-DCA
0 DA1A	0	0.311	0.320	-	-
1 DA1A	1	0.374[1]	0.487[2,3]	0.005	-
3 DA1A	3	0.329	0.674	0.028	-
7 DA1A	7	0.053	0.533	0.036	-
-1 DA2A	14	0.011	0321	0.017	0.004
0 DA2A	15	0.111	0.507	0.021	-
1 DA2A	16	0.272[4]	0.828[5]	0.042	0009
3 DA2A	18	0.190	1.070	0.029	-
7 DA2A	22	0.128	0.696	0.026	0.003
14 DA2A	29	0.004	0.046	0.016	0.009
-1 DA3A	44	-	-	-	-
0 DA3A	45	0.042	0.130	-	-
1 DA3A	46	0.013	0.192	-	-
3 DA3A	49	0.017	0.213[6]	0.004	-
7 DA3A	53	-	-	-	-
14 DA3A[7]	60	-	-	0.004	0.003
30 DA3A	76	-	-	-	-
-1 DA4A	135	-	-	-	-
0 DA4A	136	0.200	0.283	-	-
7 DA4A[8]	143	0.042	0.186	0.017	-
14 DA4A	150	0.019	0.114	0.008	-
30 DA4A	166	-	0.003	-	-

1) 2,4-D 2-EHE found in the 15-30 cm soil layer at a mean concentration of 0.025 ppm.
2) 2,4-D found in the 15-30 cm soil layer at a mean concentration of 0.020 ppm.
3) 2,4-D found in the 30-45 cm soil layer at a mean concentration of 0.001 ppm.
4) 2,4-D 2-EHE found in the 15-30 cm soil layer at a mean concentration of 0.010 ppm.
5) 2,4-D found in the 15-30 cm soil layer at a mean concentration of 0.014 ppm.
6) 2,4-D found in the 15-30 cm soil layer at a mean concentration of 0.001 ppm.
7) Actually occurred at 16 DA2A due to heavy rainfall preventing sampling at the 14 DA2A time.
8) Sampling was meant to occur at days 1 and 3 following application, however, prolonged rain activity meant these samples could not be taken.

Table A2.27: Residue Formation and Decline, Bare Soil (ppm) – Corn Use Pattern, 7 of 7.

Time	Cumulative	2,4-D 2-EHE	2,4-D	2,4-DCP	2,4-DCA
0 DA1A	0	0.415	0.622	-	-
1 DA1A	1	0.577[1]	0.837[2]	-	-
3 DA1A	3	0.256	0.501[3]	-	-
7 DA1A	7	0.250[4]	0.761[5]	0.015	-
-1 DA2A	18	0.155	0.943	0.016	-
0 DA2A	19	0.238	0.863	0.009	-
1 DA2A	20	0.284	0.820	0.009	-
3 DA2A	22	0.297[6]	0.945[7]	0.014	-
7 DA2A	26	0.189[8]	1.086[9]	0.018	-
14 DA2A	33	0.032	1.237[10]	0.027	-
-1 DA3A	52	-	0.01	-	-
0 DA3A	53	0.056	0.149	-	-
1 DA3A	54	0.048	0.253	-	-
7 DA3A	60	-	0.004	-	-
14 DA3A	67	-	-	-	-
-1 DA4A	141	-	-	-	-
0 DA4A	142	0.125	0.501	-	-
1 DA4A	143	0.147[11]	0.495[12]	-	-
3 DA4A	145	0.057	0.827	-	-
7 DA4A	149	0.061	0.734[13]	-	-
14 DA4A	156	0.004	0.478	-	-
30 DA4A	172	-	0.028	-	-

1) 2,4-D 2-EHE found in the 15-30 cm soil layer at a mean concentration of 0.068 ppm.
2) 2,4-D found in the 15-30 cm soil layer at a mean concentration of 0.099 ppm.
3) 2,4-D found in the 15-30 cm soil layer at a mean concentration of 0.036 ppm
4) 2,4-D 2-EHE found in the 15-30 cm soil layer at a mean concentration of 0.004 ppm.
5) 2,4-D found in the 15-30 cm soil layer at a mean concentration of 0.007 ppm.
6) 2,4-D 2-EHE found in the 15-30 cm soil layer at a mean concentration of 0.010 ppm.
7) 2,4-D found in the 15-30 cm soil layer at a mean concentration of 0.027 ppm.
8) 2,4-D 2-EHE found in the 15-30 cm soil layer at a mean concentration of 0.035 ppm.
9) 2,4-D found in the 15-30 cm soil layer at a mean concentration of 0.165 ppm.
10) 2,4-D found in the 15-30 cm soil layer at a mean concentration of 0.020 ppm.
11) 2,4-D 2-EHE found in the 15-30 cm soil layer at a mean concentration of 0.004 ppm.
12) 2,4-D found in the 15-30 cm soil layer at a mean concentration of 0.004 ppm.
13) 2,4-D found in the 15-30 cm soil layer at a mean concentration of 0.004 ppm.

Findings: **Half-lives**

Least squares linear regression was performed with both the 2,4-D 2-EHE and 2,4-D, calculated for dissipation curves using the slope derived from the regression model in the equation T1/2 = -ln(2)/m.

Table A2.28: Half-life (days) and r^2 Values From Seven Bare Ground Field Dissipation Studies.

	Study	1		2		3		4		5		6		7	
		$T_{1/2}$	r^2	$T_{1/2}$	r^2	$T_{1/2}$	r^2	$T_{1/2}$	r^2	$T_{1/2}$	r^2	$T_{1/2}$	r^2	$T_{1/2}$	r^2
2-EHE	App 1	2.7	0.90	1.7	0.99	0.9	0.94	4.4	0.91	12.9	0.21	2.9	0.95	10.9	0.76
	App 2	2.6	0.85	1.4	0.94	0.5	0.99	3.6	0.61	5.2	0.75	2.6	0.78	4.6	0.85
	App 3											2.9	0.35	1.2	0.99
	App 4											4.1	0.97	2.9	0.90
2,4-D	App 1	4.4	0.84	6.0	0.95	2.7	0.86	6.1	0.82	6.0	0.95	42.3	0.08	NA	NA
	App 2	15.0	0.72	2.0	0.93	1.8	0.94	5.6	0.98	**3.2**	0.91	3.7	0.7	5.5	0.79
	App 3											NA	NA	1.2	0.94
	App 4											**4.4**	0.92	7.0	0.79

NA – No calculation possible. Values in bold are calculated by DEH.

Study 6 of 7: 2,4-D residues increased after each application for around 3 days prior to declining. The half-lives reported above are from residues detected from the days of application, hence the generally poor correlation coefficients following applications 1 and 2. A half-life could not be determined for application 3 as there were only residues found (in an increasing fashion) for the first three days following the application. By day 7, no residues were detected; therefore, the half-life must be less than 7 days. The half-life for 2,4-D following the fourth application has been calculated by DEH as that reported in the test report did not account for residues found 30 days after this application. If the rate of decline following applications 1 and 2 is considered after 2,4-D reaches its peak, the half-lives are 2.8 and 0.6 days respectively with corresponding r^2 values of 0.999 and 0.96.

Study 7 of 7: 2,4-D residues increased for 14-17 days following the first and second applications. Consequently, no half-life for dissipation was possible for the 1st application as the second was made 19 days later and no decline had been observed. Based on observations from the 2nd application, once residues of 2,4-D peak, the half-life for dissipation is rapid.

Conclusions:

Mobility of all analytes tested was generally retained to the top 15 cm of soil although on occasions significant movement through the soil column was observed.

The half-life of 2,4-D 2-EHE in the seven bare ground trials varied with a range of 0.5 days to 12.9 days. The majority of dissipation half-lives ranged from around 1-5 days. Of the 18 half-lives determined, the mean half-life was 3.8 days and the 90th percentile half-life was determined to be 6.9 days.

2,4-D residues in many of the tests increased in concentration as the parent was converted to the acid before decreasing. The period of residue formation varied among tests, but was generally in the area of 3-7 days before residues reached a maximum level. In one test, this time was considerably longer with residues not appearing to peak in 19 days following the first application and around 14 days following the second application.

The half-life of 2,4-D in the seven bare ground trials varied with a range of 1.2 days to 42.3 days. All except two of the dissipation half-lives were between 1 and 7 days. Of the 15 half-lives determined, the mean half-life was 7.3 days and the 90th percentile half-life was determined to be 11 days.

Aquatic

No aquatic field dissipation studies were submitted to the APVMA. The US EPA has assessed an aquatic field dissipation study for 2,4-D BEE (MRID number 42574701). This study has not been provided to the APVMA but will be considered in the overview report.

Avian Toxicity

Acute

Three studies were provided to the APVMA for review, and one result was obtained from the ECOTOX database with the following results:

Table A2.29. Summary of Acute Bird Toxicity Results for 2,4-D Esters

Test substance	Species	LD50 (mg ae/kg bw)	Reference
2,4-D EHE	Mallard duck[1]	423	Beavers, 1984a
2,4-D EE[2]	Domestic chicken	1759	Chittibabu, 2002c
2,4-D EE[2]	Pigeon	346	Chittibabu, 2002d
2,4-D BE	Mallard duck	>4640	ECOTOX Database

1) US EPA, 2005 notes this study (based on the reported MRID number) being with Bobwhite quail.
2) Non-Standard test.

The following studies were provided to the APVMA for review:

Test Substance: **2,4-D EHE (Iso octyl ester – 63.84% ae)**

Report: Beavers, 1984a

Guidelines: FIFRA Guideline 71-1

GLP: yes

Test System

The study aimed to evaluate the acute toxicity of 2,4-D EHE when administered to mallard ducks (*Anas platyrhynchos*) as a single oral dose. Six groups of 10 birds were assigned to each treatment and control group. The age of the ducks was not provided although it is stated they were mature. 5 males and 5 females were included in each group with each sex in a separate pen. Nominal test concentrations were 0 (control), 259, 432, 720, 1200 and 2000 mg/kg bw. The test substance was dispersed in corn oil.

The primary phases of the study were an acclimation period (at least 2 weeks), a fasting period of 15 hours prior to dosing, dosing (experimental start), and post dosing observation of 14 days. During the test, the average temperature was around 23^{o}C and the average relative humidity was around 80%. The photoperiod was 8 hours of light per day through the study.

All birds were observed daily from test initiation until study termination for signs of toxicity and abnormal behaviour along with mortality. Individual body weights were measured at days 0, 3, 7 and 14 while average estimated feed consumption was determined for each group and control for days 0-3, 4-7 and 8-14. The LD50 value was statistically determined using probit analysis.

Findings

Mortalities of 0, 0, 30, 70, 90 and 80% were recorded for the control, 259, 432, 720, 1200 and 2000 mg/kg bw levels respectively. In the 432 mg/kg bw group, the first two deaths were recorded at day 1 with the third at day 2. A similar pattern was found in the higher three levels where all recorded deaths occurred within days 0 or 1 following dosing (except one death at day 3 in the 720 mg/kg bw group).

Symptoms of toxicity were observed at all dosage levels and typically included hyperexcitability, lethargy, reduced reaction to external stimuli, loss of coordination, wing droop, rapid respiration and lower limb weakness. Overt signs were noted within one hour of dosing at the 259 mg/kg and 432 mg/kg dosages and within 15 minutes at the higher doses. At 432 mg/kg and above, depression and flaccid paralysis were also observed.

For days 0-3 there was a numerical loss of body weight and a reduction in feed consumption among the survivors at dosages of 432 mg/kg or greater. There was no apparent effect on body weight change or feed consumption of surviving birds when compared to the control during the remainder of the study.

Conclusion

The acute oral LD50 of 2,4-D EHE to the mallard was 663 mg/kg bw (95% CI 458-914 mg/kg bw) equating to 423 mg ae/kg bw. The NOEC was <259 mg/kg bw (<165 mg ae/kg bw) based on overt signs of toxicity.

Test Substance:	**2,4-D Ethyl Ester**
Report:	Chittibabu, 2002c
Guidelines:	Gaitonde committee Guideline (6.4.0.Di)
GLP:	No (Quality Assurance Statement provided)

Test System

The acute oral toxicity of 2,4-D Ethyl Ester (34.6% w/w 2,4-D) was assessed on the chicken (*Gallus domesticus*) following a non-standard guideline. A total of 12 birds in four groups (3 per group) were tested and birds were 8-14 weeks old weighing 1.2-1.5 kg each at the start of the test. Birds were acclimatised for 5 days prior to dosing. They were housed in single tier wire bottomed cages that were cleaned daily. Food and water were provided *ad libitum*.

The test substance was mixed with distilled water to obtain a homogenous test solution. All birds were starved overnight prior to oral intubation. Dose rates were 2500, 5000 and 7500 mg formulation/kg bw respectively, and a control group was maintained. All birds were observed daily, individually for 21 days. Body weights were recorded immediately prior to dosing then at days 7, 14 and 21. Mortality and toxicity symptoms were observed daily throughout the study. Following test termination, survivors were necropsied for gross pathological observations.

Changes in body weight gain were compared to control birds using Student's t-test. The LD50 was calculated using Finney's Probit Analysis software.

Findings:

Mortality in the control, 2500, 5000 and 7500 mg/kg bw groups was 0, 0, 1 and 3 birds respectively corresponding to 0, 0, 33.3 and 100% respective mortality. The only death in the 5000 mg/kg bw group occurred on day 7, while in the highest

treatment group, 1 bird died on day 6, 9 and 14. Birds treated with the highest rate exhibited dullness and incoordination after 72 h while birds treated with 5000 mg/kg bw exhibited dullness alone. Birds in the lowest treatment group and control did not exhibit any signs of toxicity.

There were no significant changes in body weights between surviving birds in any of the treatment groups compared to control birds on days 7, 14 and 21. Gross pathology examination did not reveal any treatment related lesions. No abnormalities were found in the control birds.

Conclusions:

The acute oral LD50 of the 2,4-D formulation to the chicken was calculated to be 5085 mg/kg bw with the confidence limits (assumed to be 95%) ranging from 3681-6488 mg/kg bw. In terms of acid equivalence, given the level of 2,4-D in the test formulation, the LD50 was 1759 mg ae/kg bw.

Test Substance:	**2,4-D Ethyl Ester**
Report:	Chittibabu, 2002d
Guidelines:	Gaitonde committee Guideline (6.4.0.Di)
GLP:	No (Quality Assurance Statement provided)

Test System

The acute oral toxicity of 2,4-D Ethyl Ester (34.6% w/w 2,4-D) was assessed on the pigeon (*Columba livia*) following a non-standard guideline. A total of 12 birds in four groups (3 per group) were tested and birds were 8-14 weeks old weighing 220-250 g each at the start of the test. Birds were acclimatised for 5 days prior to dosing. They were housed in single tier wire bottomed cages that were cleaned daily. Food and water were provided *ad libitum*.

The test substance was mixed with distilled water to obtain a homogenous test solution. All birds were starved overnight prior to oral intubation. Dose rates were 500, 900 and 1620 mg formulation/kg bw respectively, and a control group was maintained. All birds were observed daily, individually for 21 days. Body weights were recorded immediately prior to dosing then at days 7, 14 and 21. Mortality and toxicity symptoms were observed daily throughout the study. Following test termination, survivors were necropsied for gross pathological observations.

Changes in body weight gain were compared to control birds using Student's t-test. The LD50 was calculated using Finney's Probit Analysis software.

Findings:

Mortality in the control, 500, 900 and 1620 mg/kg bw groups was 0, 0, 1 and 3 birds respectively corresponding to 0, 0, 33.3 and 100% respective mortality. The only death in the 500 mg/kg bw group occurred on day 6, while in the highest treatment group, 2 birds died on day 6 and 1 on day 7. In this highest treatment group, birds exhibited dullness, incoordination and ruffled feathers after 48 hours, while birds treated with 900 mg/kg bw exhibited dullness alone. Birds in the lowest treatment group and control did not exhibit any signs of toxicity.

There were no significant changes in body weights between surviving birds in any of the treatment groups compared to control birds on days 7, 14 and 21. Gross

pathology examination did not reveal any treatment related lesions. No abnormalities were found in the control birds.

Conclusions:

The acute oral LD50 of the 2,4-D formulation to the pigeon was calculated to be 998.9 mg/kg bw with the confidence limits (assumed to be 95%) ranging from 717-1280 mg/kg bw. These values are reported in terms of test product. Therefore, the acid equivalent LD50 is 346 mg ae/kg bw.

Test Substance: **2,4-D Butyl ester (CAS 94-80-4)**

Reference: http://www.epa.gov/ecotox/).

Mallard ducks (*Anas platyrhynchos*) 14 days of age were exposed for duration of 8 days to an application of 2,4-D Butyl ester through the oral exposure route via a capsule. No further details are available except the LD50 was >4640 mg/kg body weight.

Apart from data provided to the APVMA for review, the US EPA assessed several 2,4-D ester studies for bird oral toxicity. No comment can be made on these studies as they have not been assessed by DEH. However, the results provided by the US EPA are as follows:

Table A2.30: Additional Acute Avian Toxicity Data Reported in US EPA, 2005.

Species	LD50 (mg ac/kg)	LD50 (mg ae/kg)	MRID No.
2,4-D 2-EHE			
Mallard duck *(Anas platyrhynchos)*	>3000	>1980	72472
Mallard duck *(Anas platyrhynchos)*	>4640	>3062	226397
2,4-D BEE			
Northern bobwhite quail *(Colinus virginianus)*	>2000	>1380	414541-01
2,4-D IPE			
Northern bobwhite quail *(Colinus virginianus)*	1879	1578	439350-01

No comparable studies for the mallard duck were provided for 2,4-D BEE or IPE.

Short-Term

Two avian dietary studies using 2,4-D esters were provided to the APVMA and a further three experimental results were obtained from the ECOTOX database.

Table A2.31. Summary of Short-Term Bird Toxicity Results for 2,4-D Esters

Test substance	Species	LD50 (mg ae/kg diet)	NOEC (mg ae/kg diet)	Reference
2,4-D EHE	Mallard duck	>3588	1136	Beavers, 1984b
2,4-D EHE	Bobwhite quail	>3588	2017	Beavers, 1984c
2,4-D BE[1]	Mallard duck	10000 (ac)	-	ECOTOX database
2,4-D BE[1]	Bobwhite quail	12979 (ac)	-	ECOTOX database
2,4-D BE[1]	Bobwhite quail	10000 (ac)	-	ECOTOX database

1) Data not reviewed by DEH

Test Substance:	**2,4-D EHE (63.84% ae)**
Report:	Beavers, 1984b
Guidelines:	FIFRA Guideline 71-2
GLP:	yes

Test System

The study aimed to evaluate the toxicity of 2,4-D EHE when administered to juvenile mallard ducks (*Anas platyrhynchos*) in the diet for 5 days. Nominal test levels were 562, 1000, 1780, 3160 and 5620 ppm ac. The test consisted of an acclimation period of 7 days, an exposure period of 5 days and a post exposure observation period of 3 days.

The birds were 9 days of age at the initiation of the study. Birds were assigned to 5 test groups and 5 control groups. Each treatment and control group contained 10 ducklings that were not differentiated on sex due to their age. Test diets were prepared by mixing the test substance into the diet with corn oil. It does not appear that the stability of the test substance in the avian diet was studied prior to test initiation. Average ambient room temperature was around 27^{o}C and the average relative humidity was not reported. The photoperiod was 14 hours of light per day during acclimation and through the study.

Following test initiation and continuing until termination, all birds were observed daily. Observations of mortality, signs of toxicity and abnormal behaviour were recorded. Individual body weights were measured at test initiation, on day 5 and at termination of the test on day 8. Feed consumption during the exposure period and observation period was recorded for each pen. Feed consumption data were reported as an estimate due to unavoidable wastage by the birds.

Findings

No mortalities were recorded for the controls or any treatment except 20% mortality at the highest treatment level of 5620 ppm. At test concentrations of 3160 ppm or less there were no overt signs of toxicity and all birds were normal in appearance and behaviour throughout the test.

At 5620 ppm, signs of toxicity were first apparent on day 3 when the birds were noted to lethargic. On day 4, one bird displayed depression, reduced reaction to external stimuli, wing droop, loss of coordination and lower limb weakness; others displayed lethargy and reduced reaction to external stimuli. On day 5 one bird was found dead and the rest showed reduced reaction to external stimuli and lethargy. On day 6 all remaining birds appeared normal, however, on day 7, one bird was found dead.

During the exposure there was a reduction in body weight gain at 3160 ppm compared to the controls and a body weight loss at 5620 ppm. During the same period there was a reduction in feed consumption at the 3160 and 5620 ppm concentrations compared to the controls.

Conclusion

The dietary LC50 value for mallards exposed to 2,4-D EHE in the diet is greater than 5620 ppm (>3588 ae ppm). The authors state a NOEC of 1780 ppm (1136 ae ppm) based on reduction in body weight gain and feed consumption at the 3160 ppm test concentration.

Test Substance:	**2,4-D EHE (63.84% ae)**
Report:	Beavers, 1984c
Guidelines:	FIFRA Guideline 71-2
GLP:	yes

Test System

The study aimed to evaluate the toxicity of 2,4-D EHE when administered to juvenile Bobwhite quail (*Colinus virginianus*) in the diet for 5 days. Nominal test levels were 562, 1000, 1780, 3160 and 5620 ppm ac. The test consisted of an acclimation period of 10 days, an exposure period of 5 days and a post exposure observation period of 3 days.

The birds were 11 days of age at the initiation of the study. Birds were assigned to 5 test groups and 5 control groups. Each treatment and control group contained 10 ducklings that were not differentiated on sex due to their age. Test diets were prepared by mixing the test substance into the diet with corn oil. It does not appear that the stability of the test substance in the avian diet was studied prior to test initiation. Average ambient room temperature was around 37^{o}C and the average relative humidity was not reported. The photoperiod was 14 hours of light per day during acclimation and through the study.

Following test initiation and continuing until termination, all birds were observed daily. Observations of mortality, signs of toxicity and abnormal behaviour were recorded. Individual body weights were measured at test initiation, on day 5 and at termination of the test on day 8. Feed consumption during the exposure period and observation period was recorded for each pen. Feed consumption data were reported as an estimate due to unavoidable wastage by the birds.

Findings

Three mortalities were recorded in the control group, one on day 1, one on day 6 and one on day 7. No external lesions were observed on the bird found dead on day 1. The other two deaths were attributable to physical injury.

The only mortalities observed in the treatment groups was 20% mortality in the lowest treatment group (562 ppm). Of these deaths, one occurred on day 3 and the other on day 5. They are not considered to be treatment related given the lack of mortality at higher dose levels and higher control mortality. There were no overt signs of toxicity at any test concentration and all survivors were normal in appearance and behaviour throughout the test.

During the exposure period, a slight reduction in body weight gain was apparent in the 5620 ppm group (average change of 4 g/bird) compared to the control groups (average change around 7 g/bird). Following cessation of exposure, there was no difference between weight gain in any treatment group and the control groups. There was no apparent difference in feed consumption between the controls and any treatment groups either during the exposure period or the post exposure observation period.

Conclusion

The dietary LC50 value for bobwhite quail exposed to 2,4-D EHE is greater than 5620 ppm (>3588 ae ppm). The NOEC was determined to be 3160 ppm (2017 ae ppm) based on the reduction in body weight gain at the 5620 ppm test concentration.

The US EPA Ecotox database provides some further avian dietary results from tests using 2,4-D Butyl ester as follows:

Test Substance: **2,4-D Butyl ester (CAS 94-80-4)**

Reference: http://www.epa.gov/ecotox/).

Mallard ducks (*Anas platyrhynchos*) of an unspecified age were exposed for duration of 8 days to 2,4-D Butyl ester through the oral in the diet. No further details are available except the LD50 was 10000 ppm.

Test Substance: **2,4-D Butyl ester (CAS 94-80-4)**

Reference: http://www.epa.gov/ecotox/).

Bobwhite quail (*Colinus virginianus*) of an unspecified age were exposed for duration of 8 days to 2,4-D Butyl ester through the oral in the diet. No further details are available except the LD50 was 12979 ppm (95% CI 4548-37080 ppm).

Test Substance: **2,4-D Butyl ester (CAS 94-80-4)**

Reference: http://www.epa.gov/ecotox/).

Northern Bobwhite (*Colinus virginianus*) of an unspecified age were exposed for duration of 8 days to an application of 2,4-D Butyl ester through the oral exposure route in the diet. No further details are available except the LD50 was 10000 ppm.

In addition, the US EPA assessed several studies using 2,4-D esters with the following results:

Table A2.32: Additional Acute Avian Toxicity Data Reported in US EPA, 2005.

Species	5-Day LC50(mg ac/kg-diet)[1]	5-Day LC50(mg ae/kg-diet)	MRID
2,4-D 2-EHE			
Northern bobwhite quail *(Colinus virginianus)*	>10,000	>6600	45070
Mallard duck *(Anas platyrhynchos)*	>10,000	>6600	226397
2,4-D BEE			
Northern bobwhite quail *(Colinus virginianus)*	>5620	>3878	414484-01
Mallard duck *(Anas platyrhynchos)*	>5620	>3866	414290-07
2,4-D IPE			
Northern bobwhite quail *(Colinus virginianus)*	>5456	>4583	439349-01
Mallard duck *(Anas platyrhynchos)*	>5218	>4383	439352-01

1) Test organisms observed an additional three days while on untreated feed.

Conclusions for Avian Toxicity

Acute oral toxicity results were available for 2,4-D EHE, 2,4-D IPE, 2,4-D BEE, 2,4-D BE and 2,4-D EE, although only studies for 2,4-D EHE and 2,4-D EE were reviewed. Of the available results, mallard duck (LD50 = 423 mg ae/kg bw) and the pigeon (LD50 = 346 mg ae/kg bw from a non-standard test) were the most sensitive. Other results where data were not reviewed indicate the 2,4-D esters are moderately to practically non-toxic to birds, with results provided for mallard duck, bobwhite quail and the domestic chicken.

Only two short term toxicity tests were reviewed, both for 2,4-D EHE, with no defined short term LC50 able to be derived up to 3588 ppm ae for either the mallard duck or the bobwhite quail. In addition to these reviewed data, nine other results were reported, confirming this lack of toxicity through the diet. These results covered the 2,4-D EHE, 2,4-D BEE, 2,4-D IPE and 2,4-D BE esters and indicate that in general, 2,4-D esters will not be toxic to birds when consumed in the diet.

No avian reproductive tests were available for any of the 2,4-D esters.

Aquatic Toxicity

Fish – Acute

The APVMA received several studies, primarily for the 2,4-D EHE, with the following results:

Table A2.33. Summary of Acute Fish Toxicity Results for 2,4-D Esters

Test species	System	LC50 (mg ae/L)	Reference
2,4-D 2-EHE			
Rainbow trout (*S. gairdneri*)	96 h flow-through	>3.19	Alexander *et al*, 1983b
Bluegill sunfish (*L. macrochirus*)	96 h flow-through	>3.19	Alexander *et al*, 1983b
Fathead minnow (*P. promelas*)	96 h flow-through	>3.19	Alexander *et al*, 1983b
Fathead minnow (*P. promelas*)	8 d flow though	>15.88 (m)	Mayes *et al*, 1990a
Tidewater silverside (*M beryllina*)	96 h flow-through	>0.16 (m)*	Ward and Boeri, 1991a
Tidewater silverside (*M beryllina*)	96 h flow-through	>0.46 (m)*	Ward and Boeri, 1991b
Rainbow trout (*O. mykiss*)	96 h flow-through	3.12 (m)	Mayes *et al*, 1990b
Rainbow trout (*O. mykiss*)	8 d flow-through	2.03 (m)	Mayes *et al*, 1990b
2,4-D Ethyl Ester			
Mozambique tilapia (*T mossambica*)	96 h static	0.63[1]	Chittibabu, 2002m

(m) = measured concentration; * = very low recovery compared to nominal concentrations.
1) Non-standard test;

Test Material: **2,4-D EHE (Iso-octyl ester)**

Report: Alexander *et al*, 1983b

Guidelines: US EPA Guideline 72-1

GLP: no

Test system:

The acute toxicity of 2,4-D EHE (63.84% ae) was tested on three fish species, rainbow trout (*Salmo gairdneri*), bluegill (*Lepomis macrochirus*) and fathead minnow (*Pimephales promelas*) for 96 hours under apparent flow-through conditions. Fish were acclimated for at least 10 days during which time dilution water flow rate was at least 2 L/minute and a 16 h light/8 hour dark photoperiod was maintained. It is not clear whether these were also the test conditions, but this is assumed to be the case.

Standard dilution water was taken from Lake Huron and limed and flocculated with ferric chloride. The water was then carbon filtered, UV irradiated and pH adjusted with CO_2 prior to use. Prior to test initiation, the water had a pH of 8.4, hardness of 78 mg/L as $CaCO_3$ and alkalinity 51 mg/L as $CaCO_3$. Each test vessel contained 10

fish in 8 litres of water. Gentle aeration proceeded for at least 4-8 hours prior to toxicant exposure. Exposure to the test material was initiated by termination of aeration and addition of the appropriate amount of toxicant in acetone with 2 L of dilution water. Test concentrations for all species were control, acetone control, 0.65, 1.1, 1.8, 3.0 and 5.0 mg/L nominal. It does not appear any analysis was performed on dilution water to verify exposure concentrations.

The effect criterion was mortality, which was recorded daily.

Findings:

In all test concentrations some test material was observed to not be in solution. There was no mortality in any of the nominal concentrations and additionally, no sublethal effects were noted during any of the three tests. There was no control mortality except in the bluegill test; one fish died at 72 hours in the dilution water control.

Conclusion:

The solubility of 2,4-D EHE was measured for this test at around 6 ppb. However, the water was not sterile, and this compound would be expected to hydrolyse to 2,4-D acid that is considerably more soluble. The LC50s for all three species were >5.0 mg/L (>3.19 mg ae/L), the highest concentration tested. In this study, 2,4-D EHE is not considered toxic to the limit of its solubility.

Test Material:	**2,4-D 2-EHE**
Report:	Mayes *et al*, 1990a
Guidelines:	US EPA Guideline 72-4
GLP:	yes

Test system:

This 8 day acute test was performed in conjunction with the early life stage testing described below. Juvenile fathead minnows (*Pimephales promelas*) were exposed under flow-through conditions at measured concentrations of 0 (control), 0 (solvent control), 10.79 (1.56), 14.61 (11.63), 19.97 (11.88), 21.53 (10.16), and 41.38 (23.95). The numbers in brackets represent the standard deviation from the measured values, and it can be seen that actual exposure concentrations fluctuated significantly through the test. The authors suggest this is due to a heterogeneous distribution of test substance as it was present in much higher concentrations than its water solubility. There is no observation made as to whether a precipitate was observed, however, when sampling dilution waters for chemical analysis, samples were drawn up through a solid phase extraction column, so any compound available in undissolved form is likely to have been excluded from analysis.

Each test concentration and control was set in duplicate with each replicate containing 10 fish. Fish were observed daily with mortality and sub-lethal effects recorded. The test was terminated after 8 days of exposure and all control fish measured for weight and length.

Dissolved oxygen, temperature and pH were recorded from each replicate daily during the test.

Findings:

In the control water, hardness (as $CaCO_3$) and alkalinity (as $CaCO_3$) were 71-75 mg/L and 42-48 mg/L respectively. This compared favourably to those parameters in the highest test concentration with surviving fish where respective measurements were 70-72 mg/L and 41-50 mg/L. The pH was 7.4-7.6 for both control water and exposure water in the highest concentration. Temperature ranged from 21.5-22.9^{o}C and the dissolved oxygen ranged from 6.7-8.3 mg/L.

There were no treatment related effects at any concentration tested. The only mortality was at the lowed exposure concentration and was recorded at the 96 h observation period.

Conclusion:

The NOEC and 8 d LC50 are both >41.4 mg/L (>27.4 mg ae/L nominal or >15.88 mg ae/L measured), the highest concentration tested. 2,4-D EHE was not toxic up to its limit of solubility.

Test Material:	**2,4-D 2-EHE**
Report:	Ward and Boeri, 1991a
Guidelines:	US EPA FIFRA Guideline 72-3
GLP:	yes

Test system:

The acute toxicity of 2,4-D 2-EHE was tested on tidewater silverside (*Menidia beryllina*) under flow-through conditions for 96 hours. Nominal concentrations tested were control, solvent control, 0.29, 0.49, 0.78, 1.2 and 1.9 mg/L. The solvent control received 0.1 mL/L acetone. 20 fish per concentration (2 replicates of 10) were used in the experiment. Dilution water consisted of filtered seawater diluted to a salinity of 17 ppt. Flow-through conditions were such that there were 5.8 daily volume additions to each exposure chamber.

The test was conducted using 19.6 L glass aquaria with 15 L dilution water. Test conditions included water temperature of around 22°C and a 16:8 hours light:dark photoperiod. Water samples were taken replicate vessels from each test concentration at 0 and 96 hours for analysis.

All aquaria were examined daily for mortality and behavioural changes. Dead organisms were removed. Water quality parameters were measured daily in each exposure vessel until test termination or 100% mortality. The temperature in one vessel was recorded continuously during the test.

Results of the toxicity test could not be interpreted by standard statistical techniques because greater than 50% survival occurred at all test concentrations.

Findings:

Insoluble material was observed in test vessels containing the two highest tested concentrations during the final 48 h of the test. Media in these vessels was cloudy. Measured concentrations were significantly lower than nominal values and were determined following centrifugation of dilution water samples. At the 0 hour sampling analysis, measured concentrations ranged from 4-11% of nominal values while those at 96 h ranged from 5.7-12.6% nominal. Averaging the two measured

concentrations for each exposure group gave actual exposure concentrations of 0.03, 0.04, 0.054, 0.068 and 0.24 mg/L.

No sub-lethal effects were recorded. Also, no dose-dependent mortality appeared to be induced at any exposure concentration. In the solvent control and control, 96 h mortality was 5 and 10% respectively. In the 0.03, 0.04, 0.054, 0.068 and 0.24 mg/L exposure groups, mortality at 96 h was 15, 15, 0, 20 and 5% respectively.

Conclusions:

No effects were seen at the highest rate tested, so the 96 h LC50 is >0.24 mg ac/L (>0.16 mg ae/L). 2,4-D EHE was not toxic up to its level of solubility.

Test Material:	**Esteron 99 Herbicide (66.6% 2,4-D 2-EHE)**
Report:	Ward and Boeri, 1991b
Guidelines:	US EPA FIFRA Guideline 72-3
GLP:	yes

Test system:

The acute toxicity of the formulation Esteron 99 Herbicide, containing 2,4-D 2-EHE at 66.6%, was tested on tidewater silverside (*Menidia beryllina*) under flow-through conditions for 96 hours. Formulation details were not provided, however, it was reported as a viscous brown liquid. Nominal concentrations of product tested were control, solvent control, 0.45, 0.72, 1.17, 1.77 and 3.00 mg/L. The solvent control received 0.1 mL/L acetone. 20 fish per concentration (2 replicates of 10) were used in the experiment. Dilution water consisted of filtered seawater diluted to a salinity of 17 ppt with a pH of 8.0. Flow-through conditions was such that there were 9.8 daily volume additions to each exposure chamber.

The test was conducted using 19.6 L glass aquaria with 15 L dilution water. Test conditions included water temperature of around 22°C and a 16:8 hours light:dark photoperiod. Water samples were taken replicate vessels from each test concentration at 0 and 96 hours for analysis.

All aquaria were examined daily for mortality and behavioural changes. Dead organisms were removed. Water quality parameters were measured daily in each exposure vessel until test termination or 100% mortality. The temperature in one vessel was recorded continuously during the test.

Results of the toxicity test could not be interpreted by standard statistical techniques because greater than 50% survival occurred at all test concentrations.

Findings:

Test vessels containing 1.77 mg/L were cloudy at 96 hours, and vessels containing 3.0 mg/L were cloudy throughout the test. All lower concentration groups were clear throughout the test. During the study, pH ranged from 6.9-8.0, temperature from 21.2-22.8^{o}C and dissolved oxygen from 6.7-7.8 mg/L.

Measured concentrations were significantly lower than nominal. While the above nominal rates were for product, measured concentrations (average of 0 and 96 h results) for 2,4-D 2-EHE were 0.081, 0.14, 0.24, 0.36 and 0.70 mg/L.

No mortalities or sub-lethal effects were observed at any treatment level at any observation time throughout the test.

Conclusions:

No effects were seen at the highest rate tested, so the 96 h LC50 is >0.70 mg ac/L (>0.46 mg ae/L). 2,4-D EHE was not toxic up to its limit of solubility.

Test Material:	**Esteron 99 Herbicide (66.6% 2,4-D 2-EHE)**
Report:	Mayes *et al*, 1990b
Guidelines:	US EPA FIFRA Guideline 72-1
GLP:	yes

Test system:

The acute toxicity of the formulation Esteron 99 Herbicide, containing 2,4-D 2-EHE at 66.9%, was tested on rainbow trout (*Oncorhynchus mykiss*) under flow-through conditions for 8 days (192 h). Formulation details were not provided, however, it was reported as a clear liquid. Nominal concentrations of product tested were control, solvent control, 7.8, 13, 21.6, 36, 60 and 100 mg/L. 20 fish per concentration (2 replicates of 10) were used in the experiment. Dilution water was obtained from Lake Huron, limed and flocculated with ferric chloride. Prior to use, the water was sand-filtered, pH-adjusted, carbon filtered and UV irradiated.

Juvenile fish were acclimated to the test temperature of around 12^{o}C for at least 14 days. The test was conducted using covered glass aquaria with nylon, screen-covered drains that maintained water at around 4 L. An intermittent-flow proportional diluter was set to provide at least 6 volume turnovers per 24 h period. Test conditions included water temperature of around 12°C and a 16:8 hours light: dark photoperiod. Water samples were taken on days 0, 4 and 8 from all 16 aquaria for analysis of exposure concentration.

The report states that water quality parameters were measured once a week and on the final day of the test (noting this is only an 8 day test), but temperature in one vessel was recorded continuously during the test. All aquaria were examined daily for mortality and behavioural changes.

The 8 d LC50 was calculated using probit analysis.

Findings:

During the study, pH ranged from 7.4-7.7, temperature from 11.7-12.7^{o}C and dissolved oxygen was >75% of air saturation. Hardness and alkalinity (mg/L $CaCO_3$) ranged from 70-73 and 46-49 respectively.

Measured concentrations were significantly lower than nominal. While the above nominal rates were for product, measured concentrations (study averages) for 2,4-D 2-EHE were 1.4 (0.37), 2.2 (0.51), 3.2 (0.69), 5.5 (1.2), 10.2 (3.4) and 15.8 (3.3) mg/L. Standard deviations are reported in brackets. There is no observation made as to whether a precipitate was observed, however, when sampling dilution waters for chemical analysis, samples were drawn up through a solid phase extraction column, so any compound available in the undissolved form is likely to have been excluded from analysis.

Mortality data based on measured 2,4-D 2-EHE concentrations were recorded as follows:

Table A2.34: Mortality Findings for Rainbow Trout

	24 h	48 h	72 h	96 h	120 h	144 h	168 h	192 h
Control	0	0	0	0	0	0	0	0
Solvent	0	0	0	0	0	0	0	0
1.4	0	0	5	5	5	5	5	5
2.2	0	0	0	0	0	5	5	5
3.2	0	0	15	25	25	60	60	60
5.5	0	20	35	60	60	75	85	95
10.2	1	75	80	90	100	100	100	100
15.8	35	95	100	100	100	100	100	100

Sub-lethal effects such as surface swimming, lethargy and loss of equilibrium were observed at all test concentrations after 24 h exposure.

Conclusions:

The 96 h LC50 was calculated to be 3.12 mg ae/L. The 192 h LC50 was calculated as being 3 mg/L 2,4-D 2-EHE (2.03 mg ae/L). The NOEC was <1.4 mg ac/L (<0.95 mg ae/L) based on sub-lethal effects.

Test Substance: **2,4-D Ethyl Ester**

Report: Chittibabu, 2000m

Guidelines: Gaitonde Committee Guideline 6.4.0.D.ii

GLP: No (Quality Assurance Statement provided).

Test System

A non standard acute toxicity test of 2,4-D Ethyl Ester (34.6% w/w 2,4-D) was performed on the freshwater fish, Mozambique tilapia (*Tilapia mossambica*) over 96 hours under static conditions. Fish were acclimated to laboratory conditions for 10 days with feeding stopped 72 h prior to test initiation. Fish were 5-7.5 cm in length, presumably at the start of the test.

Water was analysed for pH, temperature, dissolved oxygen and total hardness once only, presumably at the beginning of the test. The test substance was mixed in well water to prepare stock solutions. It is unclear whether samples from the exposure media were checked for stability and homogeneity.

Based on the results of a range finding test, groups of fish (10 per group, 1 replicate per treatment) were exposed to 1.0, 1.5, 1.7, 2.2 and 2.8 mg product/L (nominal) along with a water control group. Observations for mortality and abnormal behaviour were made at 3 and 6 h, and thereafter, every 24 h until 96 h.

Findings:

At the time of measuring water quality parameters, pH was 7.4, temperature was 24^{o}C, dissolved oxygen was 7.6 mg/L and total hardness as $CaCO_3$ was 327 mg/L.

Cumulative mortality in the 1.0, 1.5, 1.7, 2.2 and 2.8 mg/L of 2,4-D Ethyl Ester 38% w/w EC groups after 96 hours was 0, 20, 40, 80 and 100% with no mortality observed in the control fish. Fish exposed to the highest test concentration were observed to

exhibit a loss of equilibrium and they lay laterally at the bottom of the aquaria. No other sub-lethal effects are reported.

Conclusion:

The 96 h LC50 for the Mozambique tilapia using probit analysis was calculated to be 1.82 mg/L with confidence limits (assumed to be 95%) of 1.71-1.92 mg/L. These values are expressed in the report as mg/L of 2,4-D Ethyl ester 38% w/w EC, which was shown to have a 2,4-D content of 34.6%. The resulting acid equivalent value would be an LC50 of 0.63 mg ae/L.

The ethyl and butyl esters of 2,4-D are commonly used in Australia and several test results have been obtained from the US EPA ECOTOX database. The available results have been distilled down to only include those post-1980 and obtained for the end of the study. For example, where results from a 96 hour study have also included 24 h, 48 h or 72 h end-points, only the 96 h end-point has been reported. In some instances, in excess of 10 results are available for a single species. Where multiple values are available, they are reported as the range and average, not a separate entry for each result. The full range of results including 95% confidence intervals may be obtained from http://www.epa.gov/ecotox/. Acute fish toxicity results for the 2,4-D ethyl and butyl esters are summarised as follows:

Table A2.35: Acute Fish Toxicity Data from Ecotox Database on 2,4-D Ethyl and Butyl Esters

Test species	System	Result (mg ac/L)	Comment
Butyl Ester			
Bluegill sunfish (*L. macrochirus*)	96 h static	LC50 = 0.29	Technical grade
Bluegill sunfish (*L. macrochirus*)	96 h static	LC50 = 0.30	Analytical grade
Cutthroat trout (*O. clarki*)	96 h static	LC50 = 0.49-2.83; average = 1.01	Total of 18 results obtained from database
Rainbow trout (*O. mykiss*)	96 h static	LC50 = 0.4	Technical grade
Rainbow trout (*O. mykiss*)	96 h static	LC50 = 0.8	Analytical grade
Lake trout, siscowet (*S. namaycush*)	96 h static	LC50 = 0.5-2.8; average = 1.04	Total of 14 results obtained from database
Ethyl Ester			
Medaka (*Oryzias latipes*)	48 h static	LC50 = 3.2	Technical grade
Goldfish (*Carassius auratus*)	48 h static	LC50 = 3.2	Technical graade
Carp (*Cyprinus carpio*)	48 h static	LC50 = 1	Technical grade
Bluegill sunfish (*L. macrochirus*)	48 h static	LC50 = 1.4	Technical grade

In addition, the US EPA reviewed several studies for the 2,4-D BEE and 2,4-D IPE that were not provided to the APVMA (US EPA, 2005). No comments can be made on these studies as they haven't been reviewed by DEH. The results of these, as reported in the US EPA assessment were:

Table A2.36: Additional Freshwater Acute Fish Toxicity Reported in US EPA, 2005. Results are in measured concentrations

Species	Test system	LC50 (mg ac/L)	LC50 (mg ae/L)	MRID
2,4-D BEE				
Rainbow trout *(Oncorhynchus mykiss)*	96 h Static	2.09	1.44	413538-01
Rainbow trout *(Oncorhynchus mykiss)*	96 h Static	0.65 (56 h)	0.45	00050674
Bluegill Sunfish (*L. macrochirus)*	96 h, assumed static	0.62	0.428	413538-01

Bluegill Sunfish (*L. macrochirus)*	96 h, assumed static	>100	0.69	400980-01
Fathead minnow (*P. promelas*)	96 h, assumed static	2.60	1.79	413538-01
2,4-D IPE				
Rainbow trout *(Oncorhynchus mykiss)*	96 h Static	0.69	0.58	439331-01
Rainbow trout *(Oncorhynchus mykiss)*	96 h Static	0.78	0.66	439332-01
Bluegill Sunfish (*L. macrochirus)*	96 h Static	0.31	0.26	439307-01
Bluegill Sunfish (*L. macrochirus)*	96 h, assumed static	0.21	0.26	439103-01

No estuarine or marine acute fish toxicity data were submitted for 2,4-D BEE or 2,4-D IPE. This is an area the US EPA identified as requiring further testing due to the higher toxicity of 2,4-D 2-EHE to marine species than the free acid or salt forms of 2,4-D, along with longer environmental half-lives of the ester forms. The need for such testing in Australia will be addressed in the risk assessment.

Fish – Subchronic/Chronic

The following study has been reviewed by DEH:

Test Material: **2,4-D 2-EHE**

Report: Mayes *et al*, 1990b

Guidelines: US EPA Guideline 72-4

GLP: yes

Test system:

The study was undertaken to determine the chronic toxicity of 2,4-D 2-EHE to fathead minnow (*Pimephales promelas*) embryos and larvae during an early life stage test with continuous aqueous exposure over 32 days. Embryos less than 24 h old were used for the test. The test was started by impartially distributing 20 embryos to each embryo cup (80 per treatment). Measured exposure concentrations were 0 (control), 0 (solvent control), 0.08, 0.12, 0.13, 0.22, 0.45 and 1.96 mg/L. **Note**, this test was run in conjunction with an 8 day acute test described above.

An intermittent flow proportional diluter was used for the test set to deliver at least 10 volume turnovers in the test aquaria each 24 hours. Test vessels were covered with glass and had a nylon screen-covered drain that maintained a water volume of around 1 L. Embryos were incubated in glass cups with nylon screen-covered bottoms that were suspended in a glass incubation chamber supported on glass beads. The flow from the delivery tube was directed into the incubation cup to produce an intermittent flow of water around the embryos during the incubation period.

The embryos were observed daily; dead embryos were counted and removed at each observation. Upon completion of hatching, the total number of larvae in each replicate, including those dead or deformed, was recorded. Larvae were observed at least once weekly and mortality and developmental abnormalities were recorded. The test continued 29 days post day-to-mean hatch of the controls (32 days total). At test termination, all surviving fish were measured for weight and length.

Dissolved oxygen, temperature and pH were recorded from each replicate at least once weekly. Water temperature was continuously recorded in one test chamber. Once a week, water hardness, alkalinity and conductivity were measured in the water control and the highest test concentration with surviving fish. The concentration of

the test substance in the test system was determined analytically during the conduct of the study.

The percent of embryos hatched, normal larvae at hatch, survival and unweighted replicate means of length and weight data were evaluated by the one-way analysis of variance procedure. The Dunnett's one-tailed t-test was used to compare treatment means to dilution water control means with only significant decreases at a level of 0.05 considered.

Findings:

Temperature in all test vessels ranged from 24.9-25.7^{o}C throughout the study. Dissolved oxygen concentrations ranged from 6.8-8.6 mg/L in all vessels throughout the study. The pH ranged from 7.1-7.6 in all vessels throughout the study.

Biological results are summarised in Table A2.35 below.

Table A2.37: ELS toxicity (32 d) of 2,4-D 2-EHE on Fathead Minnow

Concentration [mg ac/L]	Control	Solvent control	0.08	0.12	0.13	0.22	0.45	1.96[1]
Embryos hatched (%)	100	100	100	100	100	100	100	98.75
Normal Larvae at Hatch (%)	100	100	100	97.50	98.75	80.0*	73.75*	15.25*
32 day Larval Survival (%)	95.0	93.75	96.25	87.50	96.25	47.50*	28.75*	3.75*
Mean Wet Weight (mg)	61.80	74.45	73.00	71.90	69.58	64.90	60.85	40.75*
Mean Length (mm)	15.93	17.18	17.03	16.93	16.78	16.18	16.20	14.25*

1) this concentration had a standard deviation of 1.59 mg/L. * - Statistically different to the control.

There was a concentration related effect on normal larvae at hatch with the lowest effect concentration being 0.22 mg/L and the NOEC being 0.12 mg/L. Effects noted included scoliosis, hydropericardium, premature hatching (poorly developed and small) and lack of pigmentation. These effects may have been a consequence of an oily residue, presumably undissolved test material, which was observed coating the embryos in the higher concentrations. Survival through 32 days of exposure was affected at 0.22 mg/L and higher. The NOEC for this endpoint was again 0.12 mg/L. Growth (both weight and length indices) was affected at the highest test concentration; the NOEC for this endpoint was 0.45 mg/L.

Conclusion

The NOEC for all endpoints examined was 0.12 mg/L (0.079 mg ae/L). The MATC, calculated as the geometric mean of the NOEC and the LOEC (0.22 mg/L) is 0.16 mg/L (0.106 mg ae/L). The authors claim it is difficult to draw conclusions on the toxicological significance of these results as the test was conducted with treatment levels greatly exceeding the water solubility of the test substance. The above observations of embryos and larvae at the higher treatment levels being coated with an oily residue, presumably undissolved test substance, supports a view that observed effects are physical (eg, disruption of gas exchange processes). It is reasonable to conclude that 2,4-D EHE is not toxic to embryonic and larval stages of fathead minnow up to its limit of solubility.

No data were available for the registered ethyl ester and butyl ester forms of 2,4-D. MATC values as modelled through the US EPA ECOSAR v0.99g model are calculated to be 1.024 and 0.173 mg/L respectively. It should be noted that ECOSAR predicted fish 96 h LC50 values of around 6.8 and 2.2 mg/L respectively, both of

which are around an order of magnitude higher than the lowest experimental results discussed above. If this underestimation of the MATC were also true with the predicted values, actual MATCs for 2,4-D EE and 2,4-D BE could be around 0.1 and 0.02 ppm respectively.

In addition to data provided to the APVMA, the US EPA reviewed one chronic study on the freshwater fish, the fathead minnow that was not provided to the APVMA. No comments can be made on this study, as it hasn't been reviewed by DEH. The result as reported by the US EPA was:

Table A2.38: Additional Freshwater Fish ELS Test Under Flow-through Conditions Reported in US EPA, 2005. (Measured concentrations)

Species	NOEC/LOEC (mg ae/L)	MATC (mg ae/L)	Endpoints affected	MRID
2,4-D BEE Fathead Minnow (*Pimephales promelas*)	0.056/0.079	0.0662	Survival	413457-01

No chronic data for fresh or marine water species other than the above were provided for 2,4-D BEE or 2,4-D IPE.

Amphibians

Test Material: **2,4-D 2-EHE**

Report: Palmer and Krueger, 1997b

Guidelines: Full protocol provided

GLP: Yes

Test system:

Acute toxicity of 2,4-D 2-EHE to leopard frog tadpoles (*Rana pipiens*) during a 96 h exposure period under static test conditions was investigated. 20 tadpoles (2 replicates of 10) were exposed to nominal concentrations of 0 (control), 0.2, 0.6, 1.2, 2.5, 5.0, 10 and 15 mg ac/L. Mean measured test concentrations were determined from samples collected at the beginning and end of the test. However, due to the formation of 2,4-D through hydrolysis, measured test concentrations were based on those determined at the start of the test.

Tadpoles were held for around 17 days and acclimated to test conditions for around 53 hours prior to test initiation. During the 14 day holding period prior to the test, water temperatures ranged from 21-22.4^{o}C, the pH ranged from 8.3-8.5 and dissolved oxygen from 7.7-8.8 mg/L. The average total length at test initiation was 12 mm and average weight was 30 mg. Loading was calculated to be 0.16 g tadpole/L.

Test chambers were 9 L glass beakers containing around 1.8 L test solution with a water depth around 3.5 cm. Dilution water consisted of well water passed through a sand filter with further filtering to remove microorganisms and fine particles. The test was performed with a 16 h light per day photoperiod and a 30 minute dawn/dusk transition period. Temperature was measured continuously in one negative replicate. Dissolved oxygen and pH measurements were made in water sampled in all test chambers daily. Hardness and alkalinity were measured in the highest exposure group and control group at test initiation.

Organisms were observed for mortality and behaviour at 3, 24, 48, 72 and 96 hours. Due to the lack of mortality in this study, LC50 and NOEC values were estimated by visual interpretation of the mortality and clinical observation data.

Findings:

Mean measured concentrations (mean of two replicates measured at the start of the test) ranged from 84% nominal in the lowest test concentration to 4.7% nominal in the highest treatment. Recoveries decreased consistently as nominal concentrations increased, and actual exposure concentrations (and % of the nominal value) at the start of the test were 0.167 (84%), 0.409 (68%), 0.511 (43%), 0.724 (29%), 0.747 (15%) 0.756 (7.7%) and 0.703 (4.7%). These results suggest the solvent system allowed a maximum solubility of 2,4-D 2-EHE of around 0.75 mg/L. There is no observation made in the test report about undissolved test material or residues.

Temperature ranged from 21.5-22.5^{o}C and dissolved oxygen from 8.2-8.4 mg/L at the start of the study to 7.3-8.2 mg/L at the end of the study. The pH had a range of 8.2-8.5 in all concentrations and the control throughout the test. At the start of the test, hardness and alkalinity were 132 and 180 mg/L as $CaCO_3$ respectively.

No sub-lethal effects were reported throughout the study. Some mortality was observed, but no dose response could be determined. The highest mortality was 15% in the solvent control while the dilution water control recorded 5% mortality. 0-10% was recorded in the exposure groups.

Conclusion:

The 96 h LC50 for leopard frog tadpoles exposed to 2,4-D 2-EHE was >0.756 mg ac/L, the highest measured concentration where exposure occurred. This concentration was measured at the start of the test, and hydrolysis would be expected to lead to much lower concentrations by the end of the test. 2,4-D EHE is not expected to be toxic to amphibians up to its limit of solubility.

Aquatic Invertebrates – acute

The APVMA received several studies addressing acute toxicity of 2,4-D esters to various aquatic invertebrates with the following results:

Table A2.39. Summary of Acute Aquatic Invertebrate Toxicity Results for 2,4-D Esters

Test species	System	LC50 (mg ae/L)	Reference
2,4-D 2-EHE			
Daphnia magna	48 h static	12.38	McCarty, 1979
Daphnia magna	48 h static	2.92	Alexander *et al*, 1983b
Grass shrimp (*P. pugio*)	96 h flow through	>0.09 (m)*	Ward and Boeri, 1991c
Eastern oyster (*C. virginica*)	96 h flow through	NOEC = 0.14 (m)*	Ward and Boeri, 1991d
Eastern oyster (*C. virginica*)	96 h flow through	NOEC = 0.32 (m)*	Ward and Boeri, 1991e
Grass shrimp (*P. pugio*)	96 h flow through	NOEC = 0.98 (m)*	Ward and Boeri, 1991f
2,4-D Butyl Ester			
Daphnia magna	48 h static	8.44	McCarty, 1979

* very low recovery compared to nominal concentrations.; m = measured concentrations.

Test Material: **2,4-D Butyl Ester (BE)**
DOWANOL PIB Ester of 2,4-D (no further information on this)
2,4-D Ethylhexyl ester (iso-octyl ester) (EHE)

DOWANOL EB Ester of 2,4-D (no further information on this)

Report: McCarty, 1979

Guidelines: US EPA Guideline 72-2

GLP: no

Test system:

The above test materials were among 11 herbicides tested for acute toxicity to *Daphnia magna* Straus, under static conditions. The water used for the study was dechlorinated Lake Huron water with dissolved oxygen of 7.7 mg/L and total hardness of 100 mg/L as $CaCO_3$. Total alkalinity and pH are not reported.

First instar daphnids were exposed to nominal concentrations (mg/L) of the test substances as follows:

Test material	Nominal concentrations (mg/L)					
BE	4.20	6.50	10.00	32.00	56.00	100.00
PIB	5.60	10.00	18.00	32.00	42.00	65.00
EHE	32.00	42.00	55.00	75.00	100.00	
EB	5.60	10.00	18.00	32.00	42.00	65.00

All test substances were dissolved in acetone, and a solvent control was maintained during the test.

Exposure was for a period of 48 hours at 20°C water temperature. It does not appear that analysis was undertaken of the test water through the course of the study to verify dosing rates. 10 daphnids were added to each test beaker. Each concentration had three replicates (30 daphnids per concentration), as did the control group. Mortality data were recorded at 24 and 48 hours.

The LC50 was determined using probit analysis or the moving average method.

Findings:

No mortality was observed in the controls. 48 hour mortality findings for the test concentrations are as follows:

Table A2.40: 48 hour Mortality Findings (Nominal Concentrations)

BE		PIB		EHE		EB	
mg/L	% mortality	mg/L	% mortality	mg/L	% mortality	mg/L	% mortality
4.2	38	5.6	27	32	60	5.6	7
6.5	22	10	33	42	73	10	20
10	33	18	67	55	73	18	23
32	60	32	70	75	73	32	53
56	90	42	83	100	87	42	57
100	93	65	100			65	73

No further observations are reported in the test report. More than 50% mortality was observed at 48 hours in the EHE exposure group at the lowest concentration tested. Unfortunately, 24 hour mortality data were not calculated or commented on in the test report. The LC50 is therefore an extrapolation backwards for this test group.

Despite the test report stating a solvent control was maintained, no control mortality results were provided. It appears no standard dilution water control was maintained. There were no observations relating to insoluble test material, although experience

from other tests using 2,4-D esters (particularly EHE), suggest that at the levels of EHE tested here there is likely to have been significant insoluble material present.

Conclusions:

The 48h LC50 values with corresponding 95% confidence intervals were calculated as follows:

Test material	LC50 (mg/L)	95% Confidence Interval (mg/L)
BE	10.59	8.03-13.97
PIB	13.13	7.92-18.53
EHE	18.68	0.00-33.47
EB	32.55	25.61-43.69

These values are presumably calculated for active constituent, and have not been presented on an acid equivalent basis. Of the esters tested in this study, the 2,4-D butyl ester appears the most toxic although all results are of a similar order.

Test Material: **2,4-D EHE (Iso-octyl ester)**

Report: Alexander *et al*, 1983b

Guidelines: US EPA Guideline 72-2

GLP: no

Test system:

The acute toxicity of 2,4-D EHE (63.84% ae) was tested on *Daphnia magna* over 48 hours using a static test system. Dilution water was raw Lake Huron water and adjusted to a hardness of about 170 mg/L as $CaCO_3$ after which it was autoclaved at 121^{o}C and 124.1 kPa for 35 minutes. Water quality parameters for the daphnid dilution water at the time of the toxicity test were pH of 8.2, hardness of 163 mg/L as $CaCO_3$ and alkalinity 60 mg/L as $CaCO_3$.

The test was conducted with first instar daphnids. The brood vessels were held in an environmental chamber set to provide a 16:8 hour light:dark photoperiod and a temperature around 20^{o}C. The test substance was introduced dissolved in acetone. Exposure concentrations were control, acetone control, 0.65, 1.1, 1.8, 3.0 and 5.0 mg/L with 10 daphnids exposed to each group in triplicate (30 animals total per group).

Mortality was the end-point and was recorded at 24 and 48 hours of exposure. Toxicity data evaluated by the moving average method are reported here.

Findings:

In all test concentrations, some undissolved material was observed. Mortality findings for the controls and test concentrations are as follows:

Table A2.41: 24 and 48 h Mortality Findings (Nominal Concentrations).

Concentration (mg/L)	% Mortality	
	24 hours	48 hours
Control	0	3
Acetone control	3	7
0.65	0	3
1.1	10	20
1.8	13	23
3.0	3	20
5.0	23	57

No further observations relating to toxicity are made in the test report.

Conclusion:

The 48 h LC50 was calculated by the moving average method to be 4.58 mg/L (95% CI 3.05-7.12 mg/L). The acid equivalent 48 h LC50 is 2.92 mg ae/L. The study authors note this to be greater than the solubility level, however, hydrolysis of the compound would ultimately lead to exposure of 2,4-D acid, which is considerably more water-soluble. Nonetheless it is possible that effects were physical rather than toxicological.

Test Material: **2,4-D 2-EHE**

Report: Ward and Boeri, 1991c

Guidelines: US EPA FIFRA Guideline 72-3

GLP: yes

Test system:

The acute toxicity of 2,4-D 2-EHE was tested on the grass shrimp (*Palaemonetes pugio*) under flow-through conditions for 96 hours. Nominal concentrations tested were 0 (control, 0 (solvent control), 0.29, 0.49, 0.78, 1.2 and 1.9 mg/L. The test was conducted with a target temperature of around 22^{o}C. 20 shrimp were randomly distributed among 2 replicates of each treatment. The test was performed in 19.6 L glass aquaria containing 15 L test solution. A 16 h light photoperiod was maintained. During the 96 h exposure, the diluter was set to deliver an average 6.5 volume exchanges per day in each test vessel.

The number of surviving organisms and the occurrence of sub-lethal effects were determined daily and dead animals were removed. Water quality parameters were measured and recorded daily in each test chamber that contained live shrimp. The temperature in one test vessel was recorded continuously during the test. Water samples were collected from each replicate test vessel after 0 and 96 h exposure and pooled for analysis of test concentration.

Results of the test could not be interpreted by standard statistical techniques because greater than 50% survival occurred at all tested concentrations.

Findings:

Insoluble material was observed in all non-control test vessels during the test. Vessels containing the two highest concentrations were cloudy at the beginning of the study. At 24 h, white particulate matter was observed floating in all non-control vessels, and each vessel had a grey/white film on the bottom from 48-96 h. Measured

concentrations were significantly lower than nominal values. At the 0 hour sampling analysis, measured concentrations ranged from 4-7.6% of nominal values while those at 96 h ranged from 3.9-7.3 % nominal. Averaging the two measured concentrations for each exposure group gave actual exposure concentrations of 0.020, 0.033, 0.042, 0.046 and 0.14 mg/L.

No mortality or sub-lethal effects were recorded in any of the control or exposure vessels at any time during the study.

Conclusions:

No effects were found at the highest test concentration and the 96 h LC50 to the grass shrimp from exposure to 2,4-D 2-EHE is >0.14 mg ac/L (0.09 mg ae/L). 2,4-D EHE was not toxic up to its limit of solubility.

Test Material:	**2,4-D 2-EHE**
Report:	Ward and Boeri, 1991d.
Guidelines:	US EPA Guideline 72-3
GLP:	Yes

Test system:

Acute toxicity of 2,4-D was tested on the Eastern oyster (*Crassostrea virginica*) in a 96 h flow-through test, judged by the deposition of new shell. Oysters were around 12 months old at the test initiation. They were acclimatised for more than 10 days during which time the temperature ranged from 19.3-21.8^{o}C, salinity was maintained above 20 ppt, and dissolved oxygen was at least 7.4 mg/L. Oysters were 25-50 mm in height (long axis) at test initiation with each oyster ground to remove around 2-3 mm of shell to form a smooth edge.

Dilution water consisted of natural unfiltered seawater with salinity of 20 ppt and pH of 7.4. Test aquaria maintained a test solution volume of around 15 L and were equipped with an intermittent flow proportional diluter set to deliver approximately 26.8 volume replacements every 24 hours and 0.84 L per oyster per hour. Exposure was initiated by impartially selecting and placing 20 oysters in each test aquarium and the controls (single replicate only). Test conditions included a temperature of around 16^{o}C and a 16:8 hour light:dark photoperiod.

Concentrations tested included a dilution water control, solvent control, 0.27, 0.48, 0.78, 1.2 and 1.9 mg ac/L. The number of surviving organisms and the occurrence of sub-lethal effects were determined visually and recorded after 0, 24, 48, 72 and 96 hours. At the end of the study oysters were removed from test vessels and the longest finger of new growth was measured to the nearest 0.1 mm.

Analytical determination of test material concentration was performed on pooled samples from each test vessel at 0 and 96 h. Results could not be interpreted by standard statistical techniques because mean growth at all tested concentrations was >50% of the mean control growth.

Findings:

Insoluble material was not observed in any test vessel during the test. Measured concentrations were considerably lower than nominal values, and mean

concentrations were 0.016, 0.031, 0.046, 0.066 and 0.21 mg ac/L. Concentrations increased slightly during the exposure period.

During the toxicity test, the salinity was 20 ppt, the pH ranged from 7.3-7.4, temperature ranged from 15.3-17.7^{o}C, and dissolved oxygen from 7.2-8.6 mg/L. 100% survival occurred in the control and solvent control exposure and at all tested concentrations. No sub-lethal effects were noted.

No dose-response effect could be determined on shell deposition during the test. Compared to the control (mean shell growth of 1.68 mm), mean growth in the solvent control, 0.016, 0.031, 0.046, 0.066 and 0.21 mg/L exposure groups was 106, 94, 86, 84, 94 and 90% respectively, none of which were considered statistically significant.

Conclusions:

The 96 h NOEC for oyster shell deposition was 0.21 mg ac/L (0.14 mg ae/L), the highest concentration tested.

Test Material:	**Esteron 99 Herbicide (66.6% 2,4-D 2-EHE)**
Report:	Ward and Boeri, 1991e.
Guidelines:	US EPA Guideline 72-3
GLP:	Yes

Test system:

Acute toxicity of the product Esteron 99 Herbicide, containing 2,4-D 2-EHE at 66.6% was tested on the Eastern oyster (*Crassostrea virginica*) in a 96 h flow-through test, judged by the deposition of new shell. Juvenile oysters were acclimatised for more than 10 days. During the last 10 days of acclimation, the temperature ranged from 18.0-21.5^{o}C, salinity was maintained at 20 ppt, and dissolved oxygen was at least 7.4 mg/L. Oysters were 25-50 mm in height (long axis) at test initiation with each oyster ground to remove around 2-3 mm of shell to form a smooth edge.

Dilution water consisted of natural unfiltered seawater with salinity of 20 ppt and pH of 7.4. Test vessels consisted of 19.6 L aquaria that maintained a test solution volume of around 15 L and were equipped with an intermittent flow proportional diluter set to deliver approximately 23.1 volume replacements every 24 hours and 0.72 L per oyster per hour. Exposure was initiated by impartially selecting and placing 20 oysters in each test aquarium and the controls (single replicate only). Test conditions included a temperature of around 19^{o}C and a 16:8 hour light:dark photoperiod.

Concentrations tested included a dilution water control, solvent control, 0.42, 0.75, 1.2, 1.8 and 3.0 mg/L product. The number of surviving organisms and the occurrence of sub-lethal effects were determined visually and recorded after 0, 24, 48, 72 and 96 hours. At the end of the study oysters were removed from test vessels and the longest finger of new growth was measured to the nearest 0.1 mm.

Analytical determination of test material concentration was performed on pooled samples from each test vessel at 0 and 96 h. Results could not be interpreted by standard statistical techniques because mean growth at all tested concentrations was >50% of the mean control growth.

Findings:

Insoluble material was present in all test vessels containing the test material during the entire study. Measured concentrations were considerably lower than nominal values. Mean measured exposure concentrations were 0.038, 0.096, 0.22, 0.37 and 0.71 mg product/L, equating to 0.026, 0.065, 0.14, 0.24 and 0.48 mg 2,4-D 2-EHE/L.

Water quality parameter measurements showed salinity remaining at 20 ppt throughout the study, while pH ranged from 7.3-7.8, dissolved oxygen from 6.4-7.0 mg/L and temperature from 18.1-19.6^{o}C.

The test material was not toxic to oysters at any tested concentration and growth at all tested concentrations was at least 97% of the control growth.

Conclusions:

The 96 h NOEC for oyster shell deposition following exposure to 2,4-D 2-EHE in a formulation was 0.48 mg ac/L (0.32 mg ae/L), the highest concentration tested. It can be concluded that 2,4-D EHE is not toxic to oysters up to its limit of solubility.

Test Material:	**Esteron 99 Herbicide (66.6% 2,4-D 2-EHE)**
Report:	Ward and Boeri, 1991f
Guidelines:	US EPA FIFRA Guideline 72-3
GLP:	yes

Test system:

The acute toxicity of the formulation Esteron 99 Herbicide, containing 2,4-D 2-EHE at 66.6%, was tested on the grass shrimp (*Palaemonetes pugio*) under flow-through conditions for 96 hours. Nominal concentrations of product tested were 0 (control, 0 (solvent control), 0.45, 0.78, 1.17, 1.77 and 3.00 mg/L. The test was conducted with a target temperature of around 22^{o}C. Dilution water was filtered seawater adjusted to a salinity of 11-17 ppt. During the 14 days prior to testing, water temperature ranged from 20.8-22.3^{o}C and dissolved oxygen was above 6.0 mg/L.

20 shrimp were randomly distributed between 2 replicates of each treatment. The test was performed in 19.6 L glass aquaria containing 15 L test solution. A 16 h light photoperiod was maintained. During the 96 h exposure, the diluter was set to deliver an average 7.1 volume exchanges per day in each test vessel.

The number of surviving organisms and the occurrence of sub-lethal effects were determined daily. Water quality parameters were measured and recorded daily in each test chamber that contained live shrimp. The temperature in one test vessel was recorded continuously during the test. Water samples were collected from each replicate test vessel after 0 and 96 h exposure and pooled for analysis of test concentration.

Results of the test could not be interpreted by standard statistical techniques because greater than 50% survival occurred at all tested concentrations.

Findings:

Test vessels containing 3.00 mg product/L were cloudy at 48, 72 and 96 h. All lower exposure groups and the control groups were clear throughout the study. Measured concentrations of the test substance were considerably lower than nominal values. In terms of 2,4-D 2-EHE, measured concentrations were 0.035, 0.16, 0.32, 0.46 and 0.98 mg ac/L.

Salinity ranged from 14-16 ppt, pH from 7.6-7.8, dissolved oxygen from 7.6-8.3 mg/L and temperature from 21.2-22.8^{o}C throughout the test in all exposure and control vessels.

No mortality or sub-lethal effects were found in any exposure concentration or control vessel at any observation time throughout the study.

Conclusions:

No effects were found up to the highest level tested meaning the 96 h LC50 to the grass shrimp from exposure to 2,4-D 2-EHE is >0.98 mg ac/L (>0.65 mg ae/L). 2,4-D EHE is not toxic to the grass shrimp up to its limit of solubility.

The only test data for 2,4-D esters received related to the 2-ethylhexyl ester. The ethyl and butyl esters of 2,4-D are commonly used in Australia and several test results have been obtained from the US EPA ECOTOX database. The available results have been distilled down to only include those post-1980 and obtained for the end of the study. For example, where results from a 96 hour study have also included 24 h, 48 h or 72 h end-points, only the 96 h end-point has been reported. The full range of results including 95% confidence intervals may be obtained from http://www.epa.gov/ecotox/. Acute aquatic invertebrate toxicity results for the 2,4-D ethyl and butyl esters are summarised as follows:

Table A2.42: 2,4-D Butyl and Ethyl Ester Toxicity Data from the US EPA ECOTOX Database.

Test species	System	Result (mg ac/L)	Comment
Butyl Ester			
Water flea (*Daphnia magna*)	96 h static	EC50 = 2.8	Analytical grade
Stonefly (*Pteronarcella badia*)	96 h static	LC50 = 1.48	Technical grade
Stonefly (*Pteronarcella badia*)	96 h static	LC50 = 1.30	Technical grade
Ethyl ester			
Water flea (*Daphnia pulex*)	3 h static	LC50 >10	Technical grade
Water flea (*Moina macrocopa*)	3 h static	LC50 >10	Technical grade

In addition, the US EPA reviewed studies for the 2,4-D BEE and 2,4-D IPE that were not provided to the APVMA. No comments can be made on these studies as they haven't been reviewed by DEH. The results of these, as reported in the US EPA assessment were:

Table A2.43: Additional Freshwater Invertebrate Acute Toxicity Data Reported in US EPA, 2005. Measured Concentrations.

Species	Test system	LC50 (mg ac/L)	LC50 (mg ae/L)	MRID
2,4-D BEE				
Daphnia magna	48 h Static	7.2	4.97	413538-01
2,4-D IPE				
Daphnia magna	48 h Static	2.6	2.2	439306-01

Table A2.44: Additional Estuarine/Marine Invertebrate Acute Toxicity Data Reported in US EPA 2005. Measured Concentrations.

Species	Test system	LC50 (mg ac/L)	LC50 (mg ae/L)	MRID
2,4-D BEE				
Eastern oyster (*Crassostrea verginica*)	96 h	2.6	1.8	402284-01
Pink Shrimp (*Panaeus duorarum*)	96 h	5.6	3.8	402284-01

While one of the generally more sensitive species, mysid shrimp, was not tested, the US EPA is satisfied that data from the pink shrimp can be used to fulfil this requirement, and additional data will not be required at this time.

Aquatic Invertebrates - Chronic

In support of the review of 2,4-D in Australia, the following study was provided and has been reviewed by DEH.

Test Material:	**2,4-D 2-EHE (95.39% purity)**
Report:	Ward and Boeri, 1991h
Guidelines:	US EPA Guideline 72-4
GLP:	yes

Test system:

A 21 day study was undertaken on *Daphnia magna* to test the chronic toxicity of 2,4-D 2-EHE using a flow-through system. Animals were 24 h old or less at the commencement of the study. They were acclimated in well water with a hardness of 160-180 mg/L as $CaCO_3$. No preliminary test was conducted. An initial definitive test was ended because of unexpectedly poor agreement of nominal and measured concentrations, theorised to have occurred as a result of biological degradation of the test substance. A second definitive test was ended because improper cleaning of the diluter to minimise microbiological growth resulted in mortality of the test organisms. The test described here was conducted at a target temperature of around 20^{o}C at concentrations of 0 (control), 0 (solvent control), 0.11, 0.23, 0.48, 0.95 and 1.91 mg ac/L. The solvent control received 0.1 mL/L acetone.

A photoperiod of 16:8 h light:dark was maintained. The system was fitted with an intermittent flow proportional diluter designed to deliver around 26.3 volume replacements every 24 h. Each vessel contained 10 daphnids with four replicates (40 animals) per treatment level and control.

The number of surviving adult daphnids and the occurrence of sub-lethal effects were visually determined and recorded initially and at 24 h intervals. Dead animals were removed. The time to first brood was determined and the young produced by the adult daphnids were counted and removed at 1 to 3 day intervals after the onset of reproduction.

Water quality parameters were measured daily in each test chamber that contained live animals. At the termination of the test, surviving adults from each exposure cage were pooled for weighing.

Analytical determination of test concentrations was performed on days 0, 7, 14 and 21.

Results were interpreted by standard statistical techniques. The 21-Day EC50 was calculated by the binomial method.

Findings:

Water quality parameters were within acceptable levels throughout the test. Measured concentrations of dissolved oxygen were always above 60% saturation (5.6 mg/L); the temperature ranged from 19.0-21.0^{o}C and pH ranged from 7.8-8.6.

Measured concentrations were considerably lower than nominal concentrations, and actual mean exposure concentrations were established as 0.015, 0.027, 0.054, 0.089 and 0.20 mg/L. Insoluble material was not observed in any test vessel during the study.

Only the weight of surviving daphnids was statistically analysed. The 21-day survival, number of young produced and the average number of young per female was greater than the control at the concentration of test substance that was shown not to differ statistically from the control in weight.

Table A2.45 below summarises the biological results from the definitive experiment.

Table A2.45: Effects of 2,4-D 2-EHE on *Daphnia magna* in a 21 day Chronic Reproduction Study

Measured Concentration [mg ac/L]	Control	Solvent control	0.015	0.027	0.054	0.089	0.20
Parent survival at 21 days (%)	95	95	97.5	97.5	90.0	80.0	20.0
Day of first brood	8	8.5	8.5	9.2	10.5	13.2	-
Total mean offspring	1161.2	1541.8	1214.8	764.2	173.5	84.5	2.5
Mean young/surviving female	122.15	161.82	124.95	78.40	19.60	10.62	0.88
Mean body weight (mg)	0.718	0.872	0.642	0.485*	0.338*	0.300*	0.175*

* - statistically different than the control at the 95% confidence level.

The report states the weight of surviving adults was the most sensitive measured biological parameter (and this was the only parameter compared statistically). However, examination of the above data show that discernible impacts were found on all the other measured end-points. At 0.027 mg/L, parent survival was not impacted compared to the control daphnids, however, mean offspring was reduced by around 34%, with the time to first brood some 15% longer than the control daphnids.

Conclusions:

Exposure of daphnids to 2,4-D 2- EHE resulted in an MATC of 0.02 mg/L, calculated as the geometric mean of the NOEC (0.015 mg/L) and the LOEC (0.027 mg/L). The 21 day median EC50 was 0.13 mg/L (95% CI of 0.089-0.20 mg/L). In terms of acid equivalents, the 21 day median EC50 and MATC were 0.086 and 0.013 mg ae/L respectively. The NOEC was 0.01 mg ae/L.

Apart from data provided to the APVMA, the US EPA reviewed a study for 2,4-D BEE that was not provided to the APVMA. No comments can be made on this study as it hasn't been reviewed by DEH. The results of these, as reported in the US EPA assessment were:

Table A2.46: Additional Freshwater Aquatic Invertebrate Life-cycle Toxicity Data Reported in US EPA, 2005 (Measured Concentrations).

Species	21 day NOEC/LOEC (mg ae/L)	MATC (mg ae/L)	Endpoints affected	MRID
2,4-D BEE				
Daphnia magna	LC50>0.869; NOEC = 0.20;	0.311	Survival and reproduction	413538-02

	LOEC = 0.483			

No chronic data were available for other esters.

Algae and Aquatic Plants

Several studies were provided to the APVMA for the 2,4-D 2-EHE ester with the following results:

Table A2.47. Summary of Algae/Aquatic Plant Toxicity Results for 2,4-D EHE

Test species	Test duration	EC50 (mg ae/L)	Reference
2,4-D 2-EHE			
Duckweed (*L. gibba*)	14 days	0.33*	Hughes, 1990b
Green alga (*S. capricornutum*)	120 h	>1.06 (m)*	Hughes, 1990c
Marine diatom (*S. costatum*)	120 h	0.15*	Hughes, 1990d
Blue green alga (*A. flos-aquae*)	120 h	>0.21 (m)*	Hughes, 1990e
Freshwater diatom (*N. pelliculosa*)	120 h	2.7*	Hughes, 1990f

* very low recovery compared to nominal concentrations.; m = measured concentrations.

Test Material: 2,4-D 2-EHE

Report: Hughes, 1990b

Guidelines: US EPA Guideline 123-2

GLP: Yes

Test system:

A study was conducted to assess the effects of 2,4-D 2-EHE on growth inhibition to the duckweed, *Lemna gibba* over 14 days. A stock solution of 2,4-D 2-EHE was prepared by dissolving in N,N-dimethylformamide (DMF). Based on the results of a range-finding test, exposure concentrations were set at nominal values of 0 (control), 0 (solvent control), 0.0938, 0.1875, 0.375, 0.75, 1.5 and 3.0 mg ac/L. The pH of each treatment was measured. The inouclum of *L. gibba* used to begin the test was taken from 7-d old stock cultures. Three plants consisting of 4 fronds each for a total of 12 fronds, were added to each test vessel.

Incubation conditions consisted of a temperature around 25^{o}C (recorded daily). Continuous illumination was provided. Observations (frond counts) were made on test days 3, 5, 7, 10, 12 and 14. In order to eliminate subjective decisions on frond maturity, every frond visibly projecting beyond the edge of the parent frond was counted. Counts were made at approximately the same time each day.

Samples were analysed for actual test concentrations on days 0 and 14. To determine the EC25 and EC50 values and associated 95% confidence limits, weighted least squares non-linear regression of the log of test concentration against frond counts was performed. The NOEC was determined from analysis of variance and Dunnett's test. The level of significance was at 0.05.

Findings:

Detections of the test material were found only on day 0 in the 1.5 and 3.0 mg ac/L groups (limit of detection = 0.02 mg/L). At this time, the measured concentrations were 0.05 and 0.09 mg/L. 2,4-D 2-EHE was not detected for any of the other nominal

concentrations. The pH values increased throughout the study and ranged from 7.88-7.91 on day 0 and 8.91-9.66 on day 14.

Duckweed appeared far more sensitive to detection of test material than the chemical analytical method used. A summary of frond count findings on day 14 of the test is presented as follows (nominal concentrations need to be used due to very limited detections of actual concentrations):

Table A2.48: 14 day *Lemna* inhibition

Concentration (mg/L)	Solvent control	Control	0.0938	0.1875	0.375	0.75	1.5	3.0
Mean frond count (day 14)	403	561	355	327	257*	127*	90*	95*
Percent inhibition		-40.3	12.4	19.5	37.3	70.5	80.1	78.8

* significantly different from the solvent control.

Inhibition commenced with the onset of exposure. For example, after 3 days exposure (the time of first observations), inhibition in the 0.0938, 0.1875, 0.375, 0.75, 1.5 and 3.0 mg ac/L mg ac/L was 9.7, 12.9, 12.9, 16.1, 12.9 and 16.1% compared to the solvent control respectively. As the test did not continue for a recovery period, it can not be decided with any certainty whether plants would recover following cessation of exposure. While exposure even at the highest concentration did not stop frond growth (12 fronds at the start of exposure and 95 at the end), there are no observations as to the health of the plants.

Conclusion:

The lack of detection of test material is a concern. The author states this is to be expected as the concentrations tested were well above the solubility level of 2,4-D 2-EHE (of 0.03-0.05 mg/L). However, a solvent was used. In addition, there appears to be a very clear dose response to inhibition based on the above data. If *Lemna* were only exposed to the level of solubility, a similar level of inhibition could be expected at all exposure concentrations as they were all above the level of solubility. It would appear that the solvent was allowing exposure in excess of solubility. No observations are made as to cloudiness or insoluble material in the test water.

The 14 day EC25 was determined to be 0.15 mg ac/L (95% CI 0.07-0.32 mg ac/L) and the 14 day EC50 was 0.50 mg ac/L (95% CI 0.31-0.82 mg ac/L), equating to 0.33 mg ae/L. It is unclear how to interpret these results given the lack of detection of the test substance.

Test Material: **2,4-D 2-EHE**

Report: Hughes, 1990c

Guidelines: US EPA Guideline 123-2

GLP: Yes

Test system:

A study was conducted to assess the effects of 2,4-D 2-EHE to the green alga, *Selenastrum capricornutum* over 5 days. A stock solution of 2,4-D 2-EHE was prepared by dissolving in N,N-dimethylformamide (DMF). Based on the results of a range-finding test, exposure concentrations were set at nominal values of 0 (control), 0 (solvent control), 1.875, 3.75, 7.5, 15 and 30 mg ac/L. Three replicates of each concentration were performed. The pH of each treatment was measured. The

inouclum of *S. capricornutum* used to begin the test was taken from 7-d old stock cultures. Initial cell concentrations were around 3000 cells/mL.

Incubation conditions consisted of a temperature around 24°C (recorded daily). Flasks were shaken continuously. Continuous illumination was provided. Observations (cell counts) were made on test days 3, 4 and 5 using a Coulter Counter. Three counts per replicate were made.

Samples were analysed for actual test concentrations on days 0 and 5. At the end of the test, the contents of the replicate flasks were combined and the pH recorded. To determine the EC25 and EC50 values and associated 95% confidence limits, weighted least squares non-linear regression of the log of test concentration against cell counts was performed. The NOEC was determined from analysis of variance and Dunnett's test. The level of significance was at 0.05.

Findings:

2,4-D 2-EHE was only detected in the nominal concentration groups of 3.75, 7.5, 15 and 30 mg/L at respective concentrations of 1.2 (32%), 1.6 (21%), 1.0 (6.7%) and 0.5 (1.7%) mg/L at day 0. No other detections were found while the limit of detection was 0.02 mg/L. The pH values ranged from 7.11-7.21 on day 0 and 7.74-8.16 on day 5.

A summary of cell count findings on day 5 of the test is presented as follows (nominal concentrations):

Table A2.49: 5 day *Selenastrum Capricornutum* Inhibition

Concentration (mg/L)	Solvent control	Control	1.875	3.75	7.5	15	30
Day 5 cells/mL (000s)	2307	2667	2123	2070	1800*	1843	1783*
Percent inhibition		-15.6	7.9	10.3	22.0	20.1	22.7

* significantly different from the solvent control.

While the impacts on growth at 7.5 and 30 mg/L were deemed significant, it is apparent that there was little effect upon the population growth relative to the solvent control (effects were only just over 20% inhibition). Inhibition was similar for the three highest concentrations, and these all had similar measured levels at day 0, so this is not surprising. However, the nominal 3.75 mg/L group also had a similar measured concentration, but only saw 10% inhibition of growth. This indicates it is difficult to gauge any dose-response from this study.

Conclusion:

Since no test concentration resulted in more than 22.7% inhibition, the EC25 and EC50 values are greater than the highest nominal test concentration of 30 mg/L or the highest measured concentration of 1.6 mg ac/L (1.06 mg ae/L).

Test Material: **2,4-D 2-EHE**

Report: Hughes, 1990d

Guidelines: US EPA Guideline 123-2

GLP: Yes

Test system:

A study was conducted to assess the effects of 2,4-D 2-EHE to the marine diatom, *Skeletonema costatum* over 5 days. The test medium consisted of synthetic sea water (salinity 32 ppt). A stock solution of 2,4-D 2-EHE was prepared by dissolving in N,N-dimethylformamide (DMF). Based on the results of a range-finding test, exposure concentrations were set at nominal values of 0 (control), 0 (solvent control), 0.0938, 0.1875, 0.375, 0.75, 1.5 and 3.0 mg ac/L. Three replicates of each concentration were performed. The pH of each treatment was measured. The inouclum of *S. costatum* used to begin the test was taken from 7-d old stock cultures. Initial cell concentrations were around 10,000 cells/mL.

Incubation conditions consisted of a temperature around 20^{o}C (recorded daily). Flasks were shaken manually once each working day. Illumination followed a 14 h light:10 h dark photoperiod. Observations (cell counts) were made on test days 3, 4 and 5 using a Coulter Counter. Three counts per replicate were made.

Samples were analysed for actual test concentrations on days 0 and 5. At the end of the test, the contents of the replicate flasks were combined and the pH recorded. To determine the EC25 and EC50 values and associated 95% confidence limits, weighted least squares non-linear regression of the log of test concentration against cell counts was performed. Test concentrations causing growth stimulation were omitted from the regression analysis. The NOEC was determined from analysis of variance and Dunnett's test. The level of significance was at 0.05.

Findings:

The level of detection of 2,4-D 2-EHE is stated as 0.05 mg/L. On day 0, the chemical was measured in the top 4 exposure concentration, with three of these only estimated as they were below the level of detection. The nominal 0.375, 0.75, 1.5 and 3.0 mg ac/L concentrations were determined to have actual exposures of 0.04, 0.027, 0.048 and 0.08 mg ac/L respectively. No other detections at any point were made. The pH values ranged from 7.76-8.07 on day 0 and 7.02-7.17 on day 5.

A summary of cell count findings against nominal concentrations on day 5 of the test is presented as follows:

Table A2.50: 5 Day *Skeletonema costatum* Inhibition

Concentration (mg/L)	Solvent control	Control	0.0938	0.1875	0.375	0.75	1.5	3.0
Day 5 cells/mL (000s)	229	170	244	149	77*	20*	14*	15*
Percent inhibition		25.9	-6.7	35	66.6	91.1	93.8	93.5

* significantly different from the solvent control.

Effects of the test material on mean standing crop at day 5 ranged from 6.7% stimulation to almost 94% inhibition. The top three nominal exposure concentrations all resulted in similar levels of inhibition suggesting these concentrations resulted in similar amounts of test substance actually in solution. A dose-response relationship is apparent, though very uneven.

Conclusion:

The lack of detection of test material is a concern. The author states this is to be expected as the concentrations tested were well above the solubility level of 2,4-D 2-EHE (of 0.03-0.05 mg/L). However, a solvent was used. In addition, there appears to be a clear though uneven dose response to growth inhibition based on the above data. If the test diatom were only exposed to the level of solubility, a similar level of inhibition could be expected at all exposure concentrations as they were all above the

level of solubility. It would appear that the solvent was allowing exposure in excess of solubility, with similar actual exposure at the three highest nominal concentrations given the similar inhibition in these groups.

In nominal concentrations, the 5 day EC25 was determined to be 0.1 mg ac/L (95% CI 0.04-0.25 mg ac/L) and the 5 day EC50 was 0.23 mg ac/L (95% CI 0.12-0.44 mg ac/L) equating to an EC50 of 0.15 mg ae/L. The NOEC was 0.1875 mg ac/L. While the NOEC is greater than the EC25, statistical tests have determined the 35% inhibition at 0.1875 mg/L was not statistically significant.

Nominal concentrations would appear to greatly overstate actual exposure conditions, and given the lack of detection of test material, it is difficult to analyse these results.

Test Material:	**2,4-D 2-EHE**
Report:	Hughes, 1990e
Guidelines:	US EPA Guideline 123-2
GLP:	Yes

Test system:

A study was conducted to assess the effects of 2,4-D 2-EHE to the filamentous blue green alga, *Anabaena flos-aquae* over 5 days. A stock solution of 2,4-D 2-EHE was prepared by dissolving in N,N-dimethylformamide (DMF). Based on the results of a range-finding test, exposure concentrations were set at nominal values of 0 (control), 0 (solvent control), 0.938, 1.875, 3.75, 7.5, 15 and 30 mg ac/L. Three replicates of each concentration were performed. The pH of each treatment was measured. The inouclum of *A. flos-aquae* used to begin the test was taken from 7-d old stock cultures. Initial cell concentrations were around 3,000 cells/mL.

Incubation conditions consisted of a temperature around 24^{o}C (recorded daily). Flasks were shaken manually once each working day. Continuous illumination was provided. Observations (cell counts) were made on test days 3, 4 and 5 using a Coulter Counter. Three counts per replicate were made.

Samples were analysed for actual test concentrations on days 0 and 5. At the end of the test, the contents of the replicate flasks were combined and the pH recorded. To determine the EC25 and EC50 values and associated 95% confidence limits, weighted least squares non-linear regression of the log of test concentration against cell counts was performed. Test concentrations causing growth stimulation were omitted from the regression analysis. The NOEC was determined from analysis of variance and Dunnett's test. The level of significance was at 0.05.

Findings:

The limit of detection of 2,4-D 2-EHE was 0.02 mg/L. The substance was detected at all nominal concentrations on day 0, ranging from 1.1-8.5% of nominal values. There were no detections in the day 5 samples. Based on day 0 measurements, actual exposure concentrations were 0.08, 0.09, 0.19, 0.20, 0.20 and 0.32 mg ac/L. The pH values ranged from 7.37-7.58 on day 0 and 7.32-7.73 on day 5.

A summary of cell count findings on day 5 of the test is presented as follows:

Table A2.51: 5 Day *Anabaena flos-aquae* Inhibition

Measured Concentration (mg/L)	Solvent control	Control	0.08	0.09	0.19	0.20	0.20	0.32
Day 5 cells/mL (000s)	280	310	243	243	260	311	290	305
Percent inhibition		-10.6	13.3	13.1	7.1	-11.0	-3.6	-8.8

* significantly different from the solvent control.

No dose response can be determined from the above data.

Conclusion:

None of the values on day 5 in any of the test concentrations were significantly different from that in the solvent control. Therefore the highest test concentration in which growth was not significantly different from that in the solvent control is determined as the NOEC, which is 0.32 mg ac/L measured. Both the EC25 and EC50 must be >0.32 mg ac/L (>0.21 mg ae/L).

Test Material: **2,4-D 2-EHE**

Report: Hughes, 1990f

Guidelines: US EPA Guideline 123-2

GLP: Yes

Test system:

A study was conducted to assess the effects of 2,4-D 2-EHE to the non-motile freshwater diatom, *Navicula pelliculosa* over 5 days. A stock solution of 2,4-D 2-EHE was prepared by dissolving in N,N-dimethylformamide (DMF). Based on the results of a range-finding test, exposure concentrations were set at nominal values of 0 (control), 0 (solvent control), 0.1, 0.5, 1.875, 3.75, 7.5, 15 and 30 mg ac/L. Three replicates of each concentration were performed. The pH of each treatment was measured. The inouclum of *N. pelliculosa* used to begin the test was taken from 7-d old stock cultures. Initial cell concentrations were around 3,000 cells/mL.

Incubation conditions consisted of a temperature around 24^{o}C (recorded daily). Flasks were continuously shaken at 100 oscillations per minute. Continuous illumination was provided. Observations (cell counts) were made on test days 3, 4 and 5 using a Coulter Counter. Three counts per replicate were made.

Samples were analysed for actual test concentrations on days 0 and 5. At the end of the test, the contents of the replicate flasks were combined and the pH recorded. To determine the EC25 and EC50 values and associated 95% confidence limits, weighted least squares non-linear regression of the log of test concentration against cell counts was performed. Test concentrations causing growth stimulation were omitted from the regression analysis. The NOEC was determined from analysis of variance and Dunnett's test. The level of significance was at 0.05.

Findings:

The limit of detection of 2,4-D 2-EHE was stated as 0.02 mg/L. The only two measurements of the substance were at day 0 in the 15 and 30 mg/L groups where it was found at 0.062 and 0.124 mg/L respectively. No other detections were found at day 0 or day 5 measurements. The pH values ranged from 7.25-7.34 on day 0 and 7.12-7.30 on day 5 with one exception where the pH was 5.46 in the control group.

A summary of cell count findings on day 5 of the test is presented as follows:

Table A2.52: 5 Day *Navicula pelliculosa* Inhibition

Concentration (mg/L)	Solvent Control	Control	0.1	0.5	1.875	3.75	7.5	15	30
Day 5 cells/mL (000s)	1077	431	1088	1204	1099	600	162*	199*	83*
Percent inhibition		60	-1.0	-11.8	-2.0	44.3	85	81.6	92.3

* significantly different from the solvent control.

Where inhibition is compared to the control and not the solvent control, only the top three concentrations show retardation of growth, with inhibition of 62, 54 and 81% in the 7.5, 15 and 30 mg ac/L groups respectively.

Conclusion:

The day 5 cell count data show a definite increase in inhibition with increasing exposure concentration. The problem with interpreting these data lies with the lack of detection of the parent compound. Based on nominal values, the 5 day EC25 was determined to be 1.9 mg ac/L (95% CI 0.9-3.8 mg ac/L) and the 5 day EC50 was 4.1 mg ac/L (95% CI 2.5-6.7 mg ac/L) equating to an EC50 of 2.7 mg ae/kg. The NOEC was 3.75 mg ac/L, which is almost twice the EC25 value.

The only test data for 2,4-D esters received related to the 2-ethylhexyl ester. The ethyl and butyl esters of 2,4-D are commonly used in Australia and several test results have been obtained from the US EPA ECOTOX database. The full range of results including 95% confidence intervals may be obtained from http://www.epa.gov/ecotox/. The only useable result was for the butyl ester where a 3 week EC50 of 8.72 mg ac/L (95% CI 6.82-10.9) using biomass as the end point for the green algae *Selenastrum capricornutum* was reported. Many other results are available for various aquatic fungi considering reproduction effects. However, it is unclear what the end-point is without original references.

Under US EPA requirements, aquatic plant testing is required for any herbicide that has outdoor non-residential terrestrial uses that may move off-site by runoff (solubility >10 ppm in water), by drift (aerial or irrigation), or that is applied directly to aquatic use sites (except residential). At their Tier I testing level, *Kirchneria subcapitata* (formerly *Selenastrum capricornutum*) and *Lemna gibba* should be tested. Tier I testing was only performed on the green algae, *Selenastrum capricornutum* for 2,4-D esters, with most testing on aquatic plants undertaken at the Tier II level.

Table A2.53: Additional Non-target Aquatic Plant Toxicity (Tier I) Data from US EPA 2005.

Species	Dose (mg ae/L)	% Response	MRID
2,4-D IPE			
Green algae *Selenastrum capricornutum*	0.130	-11% inhibition	437680-01

The US EPA notes that this level exceeds the maximum application rate for 2,4-D acid and therefore they did not require a Tier II test.

In addition to data provided to the APVMA, additional information was reviewed by the US EPA. Under US EPA requirements, aquatic Tier II studies are required for all low dose herbicides (those with the maximum use rate of 0.5 lbs ac/A or less) and any pesticide showing a negative response equal to or greater than 50% in Tier I tests. The following species should be tested at Tier II: *Kirchneria subcapitata*, *Lemna gibba*, *Skeletonema costatum*, *Anabaena flos-aquae*, and a freshwater diatom. The following Tier II test results are available:

Table A2.54: Additional Non-target Aquatic Plant Toxicity (Tier II) Data from US EPA 2005.

Species	EC50/NOEC (mg ac/L)	EC50/NOEC (mg ae/L)	MRID
2,4-D BEE			
Duckweed *Lemna gibba*	0.58/0.204	0.40/0.14	420684-02
Green algae *Selenastrum capricornutum*	24.9/12.5	17.14/8.6	431882-01
Marine diatom *Skeletonema costatum*	1.48/0.78	1.02/0.54	420684-04
Freshwater diatom *Navicula pelliculosa*	1.86/0.86	1.28/0.59	420684-03
Blue-green algae *Anabaena flos-aquae*	6.37/3.14	4.2/2.2	420684-03

Conclusions for Aquatic Toxicity

Several acute fish toxicity results were reviewed for 2,4-D EHE and one for 2,4-D EE. Results for the former were often difficult to interpret due to inconsistent recoveries of the test substance and the main conclusion is that 2,4-D EHE is unlikely to be toxic up to its level of solubility. In one test where the ester was apparently kept in solution at sufficient quantities to obtain a result, rainbow trout appeared the most sensitive with a 96 h LD50 of 3.12 mg ae/L. The more soluble ethyl ester (2,4-D EE) was very toxic to the non-standard species Mozambique tilapia with a 96 h LC50 of 0.63 mg ae/L. Several non-reviewed results for 2,4-D EE, 2,4-D BE, 2,4-D BEE and 2,4-D IPE confirm the highly toxic nature of the 2,4-D esters to fish, both freshwater and marine. A single acute amphibian test on leopard frog tadpoles for 2,4-D EHE again resulted in a conclusion that this substance was not toxic up to its limit of solubility. One chronic study on fathead minnow with 2,4-D EHE showed a NOEC of 0.079 mg ae/L, indicating moderate toxicity.

Several acute aquatic invertebrate toxicity results were reviewed for 2,4-D EHE and one for 2,4-D BE. Again, results for the former were often difficult to interpret due to inconsistent recoveries of the test substance and the main conclusion is that 2,4-D EHE is unlikely to be toxic up to its level of solubility. Non-reviewed results for other esters included 2,4-D EE, 2,4-D BE, 2,4-D BEE and 2,4-D IPE and covered a range of aquatic invertebrates. Results for 2,4-D BE, 2,4-D BEE and 2,4-D IPE indicate these esters are moderately (approaching highly) toxic with the most sensitive result being a 96 h LC50 of 1.04 mg ae/L for 2,4-D BE to the stonefly. Two chronic test results, both to *Daphnia magna*, are available. The reviewed study for 2,4-D EHE resulted in a MATC of 0.013 mg ae/L with a 21 d EC50 of 0.036 (moderate toxicity) while the non-reviewed result for 2,4-D BEE showed this ester to be less sensitive with a MATC of 0.31 mg ae/L.

Five standard tests for different algae and the duckweed *Lemna gibba* were available for 2,4-D EHE. There were significant problems interpreting the obtained test results due to very low recoveries of test material. The marine diatom, *S. costatum*, and duckweed appeared to be the most sensitive to this ester with EC50s of 0.15 and 0.33 mg ae/L respectively (highly toxic). Generally, 2,4-D EHE did not appear toxic up to its limit of solubility. The same species were tested with 2,4-D BEE (reports not reviewed), but showed this ester to be less toxic to algae. Again, the marine diatom, *S. costatum* was the most sensitive algae with an EC50 of 1.02 mg ae/L. Duckweed was the most sensitive species overall with an EC50 of 0.4 mg ae/L, indicating it to have around the same level of toxicity to this species as 2,4-D EHE.

Terrestrial Toxicity

Non-Target Invertebrates

Bees

Three studies were submitted to the APVMA for review with the following results:

Table A2.55. Summary of Toxicity to Bees for 2,4-D Esters

Test species	Test duration	LD50 (µg ae/bee)	Reference
2,4-D 2-EHE			
Honey bee (*A. mellifera*)	72 h oral	>62.9	Hoxter *et al*, 1997a
Honey bee (*A. mellifera*)	48 h contact	>64.3	Palmer and Krueger, 1997d
2,4-D Ethyl Ester			
Worker bee (*A. indica*)	24 h contact	0.013% v/v*	Jeyalakshmi, 2002b

* Non-standard test

Test Material: **2,4-D 2-EHE**

Report: Hoxter *et al*, 1997a

Guidelines: EPPO Guideline No. 170

GLP: Yes

Test system:

The study was undertaken to evaluate the acute oral toxicity of 2,4-D 2-Ethylhexyl Ester administered to the honey bee (*Apis mellifera*) in the diet. Honey bees were exposed to a geometric series of test concentrations or 0 (control), 0 (solvent control), 6.25, 12.5, 25.0, 50.0 and 100 µg/bee in a sucrose diet. As a positive control groups of bees were also exposed to dimethoate at 0.05, 0.15 and 0.45 µg/bee.

Test chambers were stainless steel cylinders measuring around 9 cm in diameter and 9 cm high with perforations for ventilation. Each end of the cylinder was covered with a petri dish. A glass feeding tube containing a precise amount of treated or control diet was inserted through the lid of each cylinder. 20 bees were placed in each test chamber following immobilisation. Three replicates per treatment were used. The feeding tubes were monitored periodically for up to five hours from test initiation. After the dose was consumed, the tube was replaced with untreated sucrose solution. All doses were consumed within the five hour time period. It is assumed all bees in each container received a similar dose. The bees were maintained under continuous darkness except for periods of dosing and observations.

During the test, the bees were maintained at around 28-32^{o}C with relative humidity above 50%. Temperature and humidity were measured twice daily. The bees were observed periodically to evaluate mortalities and sub-lethal effects. Observations were made at around 1, 3.5, 24, 48 and 72 hours.

The LD50 value was calculated by the moving average method in the positive control group. The pattern of mortality in the treatment groups did not facilitate the calculation of LD50s, therefore, the LD50 values were estimated by visual inspection, as was the NOEC.

Findings:

During the test, the temperature ranged from 27.1-27.4^{o}C while relative humidity range from 54-88%. Based on diet remaining after the 5 hour feeding time, administered doses were 5.94, 12.5, 22.9, 50.0 and 100 μg/bee. There did not appear to be any aversion to the treated diets since treatment diet consumption was at least equivalent to the control diet consumption.

After 72 hours, mortality/immobility in the negative control and solvent control groups was 8 and 28% respectively. The large mortality in the solvent control group was first noticed on day 2. It was primarily concentrated in one replicate and was therefore considered to be incidental. In two of the three replicates in the solvent control, no deaths were observed, although one bee demonstrated lethargy and another, a loss of equilibrium. All other bees in the control groups remained normal in appearance and behaviour throughout the test.

In the positive control, 5, 85 and 98% group mortality were observed in the 0.05, 0.15 and 0.45 μg/bee levels respectively.

Based on group mortality (60 bees per treatment level in 3 replicates of 20), mortality after 72 hours in the 6.25, 12.5, 25.0, 50.0 and 100.0 μg/bee levels were 5, 5, 15, 18 and 35% respectively. Closer inspection of these data show that mortality is probably not dose-responsive. In the two highest treatments, the mortality was concentrated to one replicate. The other two replicates at both these treatment levels only showed 5-10% mortality after 72 hours.

Sublethal effects included loss of equilibrium and lethargy. These effects were generally exhibited by only a small number of bees. The only sublethal effects found at 72 hours were a loss of equilibrium in one bee of one replicate in the 6.25, 12.5 and 50.0 μg/bee treatment levels. Given no sub-lethal effects were observed at this time in the 25.0 and 100.0 μg/bee levels, it is difficult to conclude these effects are treatment related.

Conclusion:

No treatment related effects were seen at 72 hours and the 72 h acute oral LD50 is >100 μg ac/bee (>62.9 μg ae/bee), the highest rate tested.

Test Material:	**2,4-D 2-Ethylhexyl Ester**
Report:	Palmer and Krueger, 1997d
Guidelines:	US EPA Guideline 141-1; EPPO Guideline No. 170
GLP:	Yes

Test system:

The study was undertaken to evaluate the acute contact toxicity of 2,4-D 2-EHE administered to the honey bee (*Apis mellifera*). Honey bees were exposed to a geometric series of test concentrations or 0 (control), 0 (solvent control), 6.25, 12.5, 25.0, 50.0 and 100 μg/bee administered topically in a droplet to the abdomen and/or thorax of each bee. As a positive control groups of bees were also exposed to dimethoate at 0.05, 0.10 and 0.20 μg/bee. Three replicate test chambers were maintained in each treatment and control group with 20 bees in each chamber. Bees were 1-6 days of age at test initiation and were apparently healthy.

Test chambers were stainless steel cylinders measuring around 9 cm in diameter and 9 cm high with perforations for ventilation. Each end of the cylinder was covered with a petri dish. A glass feeding tube containing a sucrose diet was inserted through the lid of each cylinder. The bees were maintained under continuous darkness except for periods of dosing and observations.

During the test, the bees were maintained at around 28-32^{o}C with relative humidity above 50%. Temperature and humidity were measured twice daily. The bees were observed periodically to evaluate mortalities and sub-lethal effects. Observations were made at around 1.75, 4, 24 and 48 hours.

The LD50 values were calculated by the binomial method in the positive control group. The pattern of mortality in the treatment groups did not facilitate the calculation of an LD50, so this was estimated by visual inspection, as was the NOEC.

Findings:

During the test, the temperature ranged from 27.2-27.5^{o}C while relative humidity range from 48-81%. At test termination there was 5% mortality among bees in the negative control group, and 2% among bees in the solvent control group. All surviving bees in these two groups remained normal in appearance and behaviour throughout the test period. In the positive control, mortality was 5, 7 and 90% in the 0.05, 0.10 and 0.20 µg/bee treatments respectively.

Based on group mortality (60 bees per treatment level in 3 replicates of 20), mortality after 48 hours in the 6.25, 12.5, 25.0, 50.0 and 100.0 µg/bee levels were 2, 2, 8, 8 and 35% respectively. With the exception of three bees in the highest group that exhibited either lethargy or loss of equilibrium, all other surviving bees remained normal in appearance and behaviour throughout the test period. Only mortality at the highest level was considered treatment related as mortality in the other treatment groups was similar to control levels.

Conclusion:

The 48 h acute contact LD50 for honey bees exposed to 2,4-D 2-EHE was determined to be >100 µg ac/bee (>64.3 µg ae/bee). The NOEC was 50 µg/bee (32.1 µg ae/bee).

Test Material:	**2,4-D Ethyl Ester (38% EC)**
Report:	Jeyalakshmi, 2002b
Guidelines:	Gaitonde Committee Guideline No. 6.6.0
GLP:	No (Quality Assurance Statement provided).

Test system:

Toxicity of 2,4-D Ethyl Ester formulation (34.6% w/w 2,4-D) to worker bees (*Apis indica*), 5-15 days old, was tested using a dry film method. Thirteen treatments were performed with test product at 0.01, 0.02, 0.04, 0.06, 0.08 and 0.10% v/v, endosulfan as a toxic standard at 0.006, 0.008, 0.011, 0.014, 0.017 and 0.020% v/v, and a water control. These concentrations were based on the results of pilot studies. Three replicates of each treatment were performed with 10 bees per replicate.

For pre-conditioning, bees collected from hives were kept in glass jars covered with muslin cloth. A cotton swab soaked in 50% sugar was placed inside for feed. The jar

was kept at room temperature (around 28°C) and relative humidity around 70-90% for 15 hours (of which 12 were in the dark).

For treatment, relevant concentrations of test substance were dissolved in water. One mL of each was taken in a beaker and slowly rotated until the solvent evaporated leaving a thin film of chemical at the bottom and the walls of the beaker. Bees were anaesthetised with CO_2 and transferred to the treated beakers. After 90 minutes, bees were transferred into test cages and fed on 50% sugar solution. Mortality was recorded after 24 h. The same treatment method was followed for endosulfan with water as a solvent.

The LC50 was calculated using probit analysis.

Findings:

No mortality was observed in the control group of bees. In the toxic control, mortality ranged from 23% at the lowest treatment to 97% at the highest, confirming the integrity of the test system. In the 2,4-D ester treatment groups, mortality in the at 0.01, 0.02, 0.04, 0.06, 0.08 and 0.10% v/v treatment groups after 24 hours (mean of three replicates) was 10, 27, 40, 57, 83 and 93% respectively.

Conclusion:

The LC50 for 2,4-D Ethyl Ester 38% w/w EC was calculated to be 0.039% v/v with confidence limits (assumed to be 95%) of 0.035-0.043% v/v. In terms of acid equivalence, the LC50 would be 0.013% v/v. It is very difficult to relate this to a value of toxicity in terms of µg/bee. 10 bees were exposed to 1 mL, but this was not applied directly to the bee, rather as a thin film over glass, where bees came in contact with a small proportion. Without a clear indication of the surface area the test substance was applied to, it is not possible to relate this to an application rate per hectare.

Other Arthropods

Two studies were provided as follows:

Test Material:	**2,4-D 2-EHE (564 g/L ae formulation)**
Report:	Kuhner, 1998a
Guidelines:	LOUIS/UFER, 1995
GLP:	Yes

Test system:

The study was undertaken to determine the effect of the formulation Esteron 60 (564 g ae/L) on the predatory mite *Typhlodromus pyri* in the laboratory. The application rate was based on the maximum spraying volume (0.564 kg ae/ha product) with exposure to a direct spray situation and a 5% spray drift situation.

The study aimed to assess mortality of protonymphs (around 1 day in age) and fecundity with exposure to a freshly applied dry residue on a glass surface. The mites were fed on pollen and exposure was for 14 days. Mortality and escape rate was determined for the first 7 days, subsequently sex ratio and reproduction rate of the surviving mites was determined for the second 7 day exposure period. Dimethoate was applied as a toxic standard to confirm efficacy of the test system. Each exposure group included 5 replicates containing 20 mites each.

The test substance suspension was prepared on the day of application with an automatic laboratory sprayer. After establishment of the test units, the protonymphs were placed onto the glass surface and immediately examined for vitality. At day 3 and 7, the number of dead and living mites was counted with dead mites removed. At day 7 males and females were determined. If the sex ratio was more than 2 females per male, males originating from replicates of the same treatment were added. The number of missing organisms was calculated. Eggs and juvenile mites were counted and afterwards, removed. Food was added. From days 7-14 four assessments were done with a maximum interval of 3 days. At each, the number of dead and living mites was counted. The number of missing organisms was calculated. Dead animals were removed. Eggs and juvenile mites were counted and afterwards removed. Food was added.

The experiment was performed at around 25°C with a relative humidity of 70±15%. A 16 h light and 8 h dark photoperiod was maintained.

Findings:

The results were considered valid because the average mortality in the control group was ≤20% and the average mortality in the reference group was ≥50%. Also, the average number of eggs/female in the control group exceeded 3.

At day 7, 90% of mites were alive (10% were missing) in the direct spray group with 88% alive in the 5% spray drift group. 11 mites were missing and 1 was dead in this group. This compared to 94% in the control group and 6% in the positive control. The corrected mortality therefore for the high and low groups were 4.3 and 6.4% respectively. Both results are considered to be within the natural variability of the test system. The toxic standard had 94% mortality.

Regarding fecundity, during the 7 day egg laying period, the number of offspring per female in the control was 8.0 compared to 6.6 and 8.7 in the high and low exposure groups respectively.

Based on the two endpoints of fecundity and mortality, the reduction in beneficial capacity in the high and low treatment group was 20.6% and –2.0% respectively.

Conclusion:

Based on these results, the formulation should be classified as harmless to *T. pyri* according to IOBC categories (reduction in beneficial capacity <30%) when applied at 564 g ae/ha.

Test Material:	**2,4-D 2-EHE (564 g/L ae formulation)**
Report:	Kuhner, 1998b
Guidelines:	Polgar, 1988; Mead-Briggs, 1992
GLP:	Yes

Test system:

The study was undertaken to determine the effect of the formulation Esteron 60 (564 g ae/L) on the aphid parasitoid *Aphidius rhopalosiphi* in the laboratory. The application rate was based on the maximum spraying volume (0.564 kg ae/ha) with exposure to a direct spray situation and a 5% spray drift situation.

The study aimed to assess mortality and fertility (parasitic capacity) with exposure to a freshly applied dry residue on a glass surface. The parasitoids were confined for 48 h and their condition assessed after around 0.5, 2, 24 and 48 h. After 48 h surviving females were removed from the cages and the parasitic capacity per female assessed in a fertility test. The females were offered aphids for oviposition. Counting of parasitised aphids was carried out 11 days after the start of the fertility test and compared to the control. Dimethoate was applied as a toxic standard to confirm efficacy of the test system. Each exposure group included 4 replicates containing 20 mites each.

The test substance suspension was prepared on the day of application with an automatic laboratory sprayer. After establishment of the exposure cages, the test organisms were introduced by shaking them into the cage. After 48 h exposure to the glass plates, surviving organisms were removed and females released individually to one fertility cage to parasitise aphids for a period of 24 hours. After 24 h they were removed from the fertility cage. The plants bearing the aphids were maintained at test conditions and the number of parasitised aphids counted after 11 days.

The experiment was performed at around 20^{o}C with a relative humidity of 50-85%. Lighting was continuous for the first part of the test and then followed a 16 h light:8 h dark photoperiod for the fertility component.

Findings:

The results were considered valid because the average mortality in the control group was ≤10% and the average mortality in the reference group was ≥50%.

In the control group, all adults were alive after 48 h. From a total of 40 adults of the formulation treatment groups, mortality was 100% and 12.5% in the high and low exposure groups respectively. Full mortality was found in the toxic standard.

In the fertility test, 19 females were tested in the control group. The total number of mummies developed within 11 days was 85 for the control group corresponding to 4.5 mummies per female. 15 females were tested in the low treatment group. They produced 59 mummies, resulting in 3.9 mummies per female respectively. The reduction in reproduction rate based on these results was 12.1%.

The combination of mortality and reproduction rate resulted in an overall reduction in beneficial capacity of 100 and 23.1% in the high and low treatment groups respectively.

Conclusion:

Based on these results, the formulation should be classified as harmful to *A. rhopalosiphi* according to IOBC categories when applied at 0.564 kg ae/ha and harmless at 5% of this rate, 0.028 kg ae/ha.

Earthworms

No data provided.

Soil Micro-Organisms

No data have been provided.

Non-Target Vegetation

Five tests were provided for this endpoint. Three were seedling emergence studies, two for 2,4-D EHE (one being a supplementary test) and one for 2,4-D BEE while the other two were vegetative vigour studies, one each for 2,4-D EHE and 2,4-D BEE.

Test Material:	**2,4-D 2-EHE**
Report:	Backus and Crosby, 1992a
Guidelines:	US EPA Guideline 123-1
GLP:	No – QA statement provided.

Test system:

Tier II germination and seedling emergence studies were conducted over 14 days to evaluate non-target phytotoxicity of 2,4-D EHE to 6 dicotyledonous plant species and 4 monocotyledonous plant species. Seeds were exposed to a series of doses in Petri dishes (seed germination) and field soil (seedling emergence). The highest concentration applied was equivalent to 1080 g ae/ha. In both phases of the study, each of the remaining test dosages applied was one-half of the previous rate, that is, 540, 270, 135, 67.5, 33.8, 16.9, 8.4 and 4.2 g ae/ha. In the Petri dish study, an additional 2 rates of 2.24 and 1.12 g ae/ha were tested. There were three replicates of each of the treatments, including the solvent and untreated controls.

Acetone was used as the solvent. Due to concerns with volatility, in the Petri dish test the test material was applied to filter papers and passively dried for three hours using ambient air flow only. Water was then pipetted onto the treated filter paper, and 10 seeds of each test species placed on the filter paper. The dishes were covered and sealed with Parafilm.

For the seedling emergence in soil component of the test, the growth medium was steam-pasteurised natural soil amended with 50% silica sand and supplemental nutrients. The medium had a pH of 6.5, CEC of 3.9 meq/100 g and organic matter of 1.1%. Soil in the test pots was around 7.0 cm deep. 10 seeds per species per replicate were planted in the pots, evenly spaced. Each species was planted at an empirically determined optimal depth for germination and emergence. After planting, the seedbed was lightly tamped. A small volume of screen soil was then placed over the seedbed and levelled off. Seeds were planted on the day of the test.

Percent germination, seedling emergence and effects on fresh weight were evaluated. Several statistical analytical methods were employed to assess the measured data. The NOEL was estimated using a one-way ANOVA model.

Findings:

Test concentration analysis showed the test solutions contained between 97-100% of the nominal values for both Petri dish and soil components of the study.

Seed Germination: In the seed germination component of the study, five species, mustard, onion, radish, sorghum and tomato, were only exposed to a maximum of 270 g ae/ha. The other five species, buckwheat, corn, cucumber, oat and soybean, received a maximum of 1080 g ae/ha. The test material exhibited little activity on seed germination. EC25 and EC50 values were all at least the highest rates tested. In the species exposed to the highest rate of 1080 g ae/ha, no treatment related NOELs were found. In those exposed to the highest rate of 270 g ae/ha, the NOEL value for

sorghum was 135 g ae/ha based on a 15.8% reduction in germination compared to the highest 270 g ae/ha treatment level.

Results of the preliminary Petri dish seed germination study indicated that 2,4-D 2-EHE influenced radicle length. Corn and oats were the least sensitive species with NOELs of 6.7 g ae/ha, the highest rate tested. The NOEL for buckwheat, cucumber, sorghum and tomato was 0.67 g ae/ha, the middle rate. The NOEL for mustard, onion, radish and soybean was the lowest rate tested, 0.07 g ae/ha. Germination effects were significant for mustard, radish and tomato at the high rate and for onion at both the middle and high rates. In general, the dicot species were more sensitive than the monocots; in the Petri dish however, onion (a monocot), was the species most sensitive to the test material.

Seedling Emergence: Immediately prior to test harvest, visual observations were made. Emergence in the untreated controls was at least 90% with no symptoms observed in any plants. In the solvent controls, emergence was at least 70% with a slight damping-off problem noted on cucumber. At the highest test rate, stunting and distortion was observed on mustard, radish, sorghum and tomato with stunting only also observed for buckwheat, cucumber, onion and soybean. Emergence was at least 85% for most species.

At 540 g ae/ha and below buckwheat no longer exhibited any symptoms. Stunting was observed on cucumber, onion, radish and soybean. Stunting and distortion was observed on mustard, sorghum and tomato. In addition, radish stems appeared thickened. At 270 g ae/ha no symptoms were noted on buckwheat, corn, cucumber, oats, onion and soybean; few symptoms were noted on tomato. Slight stunting was observed on mustard, radish and sorghum with some distortion also noted on the latter species. Percent emergence was generally >85% at all test rates from day 7 through day 14.

Statistical Results

The results of the statistical analysis are shown in the following table.

Table A2.56: Effects of 2,4-D EHE on vegetative vigour of ten plant species (results in g ae/ha)

		Seed Germination			Seedling Emergence			Fresh Weight		
		NOEL	EC25	EC50	NOEL	EC25	EC50	NOEL	EC25	EC50
Monocots	Onion	≥270	≥270	≥270	135*	560-1080*	≥1080	135*	270*	≥1080
	Sorghum	135	≥270	≥270	≥1080	≥1080	≥1080	270	284	693
	Corn	1080*	≥1080	≥1080	≥1080	≥1080	≥1080	≥1080	≥1080	≥1080
	Oats	≥1080	≥1080	≥1080	≥1080	≥1080	≥1080	≥1080	≥1080	≥1080
Dicots	Tomato	≥270	≥270	≥270	≥1080	≥1080	≥1080	270*	270-1080*	≤1080
	Soybean	≥1080	≥1080	≥1080	≥1080	≥1080	≥1080	≥1080	≥1080	≥1080
	Buckwheat	540	≥1080	≥1080	≥1080	≥1080	≥1080	270*	1080*	≥1080
	Mustard	≥270	≥270	≥270	67.3*	≥270*	≤540*	33.6	≥33.6	63.7
	Radish	≥270	≥270	≥270	270	430	≥1080	16.8*	67.3-135*	164
	Cucumber	1080*	≥1080	≥1080	540	1080	≥1080	540	≥540	950**

* See following discussion. Statistical values overridden. ** Large confidence limits of 153 - >1120 g ae/ha.

Emergence: There appeared to be a solvent effect on emergence in several species, and a significant solvent effect was observed on onion emergence. Emergence in the untreated control was 76.7% while that for the solvent control was 53.3%. The NOEL for onion was therefore manually calculated to be 135 g ae/ha using the

untreated control only, and the statistically generated NOEL for onion was overridden.

Mustard, onion, radish and tomato were tested a lower rates in the Petri dish bioassay than the soil test as they were believed to be more sensitive to the test material. Comparing germination (%)/emergence NOEL values from both tests, only mustard exhibited greater sensitivity in the soil bioassay.

Fresh weight: Again, because of the significant solvent effect in onion, the statistically derived NOEL was overridden and the NOEL calculated manually from the untreated control results. Radish was the most sensitive dicot and displayed a quadratic dose-response. The statistically derived NOEL (67.3 g ae/ha) and EC25 (>67.3 g ae/ha) were overridden based on examination of the data. The NOEL was believed to be 16.8 g ae/ha. The relatively small range between the EC25 and EC50 further indicates the sensitivity of radish to this material.

Low emergence in one replicate each of the untreated and solvent controls complicated analysis of tomato data. At the 540 g ae/ha level, data did not fit the dose-response curve. Tomato exhibited a 30% effect at 1080 g ae/ha and a 20% effect at 270 g ae/ha.

Because the test material elicited a 25% effect on buckwheat at 1080 g ae/ha, this is believed to be the EC25 while the NOEL was believed to be 270 g ae/ha.

The NOEL for cucumber was 540 g ae/ha. The EC25 was undefined. At 540 g ae/ha, there was a 43.4% reduction in fresh weight. However, this was not considered statistically different from the control.

Conclusions:

Germination and emergence results indicate that 2,4-D 2-EHE did not inhibit seed germination in either a closed (Petri dish) or open (soil) situation. This material elicited little effect on the initial phases of plant growth. It is unclear why the sensitive indicator, radicle length in the preliminary test, was not measured in the definitive study.

The seedling fresh weight data generally correspond with the seedling emergence data. Emergence of cucumber, mustard, radish and tomato was affected as measured by NOEL values. Emergence and fresh weight response were virtually identical for buckwheat, corn, oats, onion and soybean. Inhibition of growth of radish appeared to occur independently of the growth process, via the roots or through the coleoptile or hypocotyl.

Monocot species known to be tolerant of 2,4-D 2-EHE did not exhibit effects due to soil application and several broadleaf species were tolerant as well. Only mustard and radish fresh weights were inhibited after emergence.

Test Material:	**2,4-D 2-EHE**
Report:	Backus, 1995
Guidelines:	US EPA Guideline 123-1
GLP:	No – QA statement provided.

Test system:

This test was a partial repetition of the above seedling emergence study where the dose response in cucumber was irregular, and emergence was reduced in the onion untreated and solvent controls. Therefore, based on the US EPA recommendation, the test reported here was performed to re-test cucumber and onion for determination of the NOEL and EC25.

A total of 11 concentrations were tested, 1.05, 2.1, 4.2, 8.4, 16.8, 34, 67, 135, 270, 540 and 1080 g ae/ha. In addition, a solvent control (acetone only) and untreated control were maintained.

The growing medium consisted of 50% steam pasteurised natural soil amended with 50% silica sand. Four replicates were performed with 20 cucumber or 40 onion seeds per replicate. Treatment was by a moving laboratory sprayer.

Visual observations were performed twice weekly. Seedling emergence compared to the controls was recorded at the end of the 14 day test period. Fresh weights of the aboveground portions of plants were taken and recorded at the end of the test.

The NOEL was determined using ANOVA. The EC25/EC50 was calculated from the dose/response regression equation if a significant dose/response was observed.

Findings:

For both plants tested, stunting and hormonal type effects were observed regularly and with increasing severity from 67 g ae/ha upwards. At lower concentrations, some stunting was observed in some replicates, but not consistently across treatments. The visual NOEL appears to be around 17 g ae/ha.

Percent emergence values of the two species tested indicated that onion emergence was more sensitive to the test material. The statistically generated NOEL of onion was 269 g ae/ha. The EC25 was calculated to be 500 g ae/ha and the EC50 was >1080 g ae/ha. For cucumbers, the NOEL was ≥1080 g ae/ha.

Analysis of fresh weight data showed cucumber to be more affected. The NOEL for cucumber fresh weight was 17 g ac/ha. The EC25 and EC50 were 50.4 g ae/ha and 210 g ae/ha respectively. The NOEL for onion fresh weights was 135 g ae/ha. The EC25 and EC50 was 244 g ae/ha and 623 g ae/ha respectively.

Conclusions:

Based on visual observations of stunting and hormone like effects, the NOEL for both cucumber and onions in the seedling emergence test was 16.8 g ae/ha, which is much lower than results from the initial seedling emergence study discussed above.

Test Material:	**2,4-D BEE**
Report:	Harnish, 1994
Guidelines:	US EPA Guideline 123-1
GLP:	No – QA statement provided.

Test system:

Tier II germination and seedling emergence studies were conducted over 14 days to evaluate non-target phytotoxicity of 2,4-D BEE to 6 dicotyledonous plant species and 4 monocotyledonous plant species. Seeds were exposed to a series of doses in Petri dishes (seed germination) and field soil (seedling emergence).

For the Tier II seed germination bioassay, three separate tests were conducted due to solvent effect and poor rate response. Ten seeds of each species were germinated in Petri dishes treated at a series of doses of 2,4-D BEE. The highest concentration was equivalent to a field rate of 63 g ae/ha. The remaining doses were ¼ of the preceding dose, that is (in rounded values), 16, 4, 1 and 0.25 g ae/ha. Four replicates per treatment for each species were tested. The two earlier studies were conducted with the highest rate tested as 16 g ae/ha with lower doses of 4, 1, 0.25, 0.06 and 0.016 g ae/ha, repeated once at the same concentrations due to solvent effects the first time.

For the seedling emergence bioassay, ten seeds of each species were planted in 15 cm pots containing pasteurised sandy soil. A single application of the test substance was applied to seeds and soil using a CO_2 pressurised hand-held spray applicator. Two tests were conducted at different rates to obtain a dose-response relationship. The original study conducted at six concentrations, the highest of which was 4 g ae/ha, failed to produce detrimental effects on any crop. Therefore, a continuation study was conducted at five concentrations being equivalent to 560, 280, 140, 70 and 35 g ae/ha.

Phytotoxicity was evaluated visually at days 7, 14, 21 and 28, although only the day 7 and 28 observations were shown in the test report results. In the seed germination component of the study, germination and radicle length were recorded. Seeds were considered germinated when the radicle was at least 4 mm in length.

In the seedling emergence component of the study, plant number and height were recorded at days 14 and 28, plants within the treated replicates were harvested at day 28 and dry weights recorded to determine the NOEC using replicate means, and seedling emergence was recorded at days 7, 14 and 21 days following treatment with the day 28 data used to evaluate effects on plant survival.

The 28 day data were used to evaluate effects and determine the NOEC. All raw data were analysed by ANOVA. If significant differences to the control were found at P = 0.05 (using F-test), then the NOEL was found by utilising an LSD mean separation test. The EC25 and EC50 were obtained using regression analysis.

It is important to note for the following discussion that for the statistical analysis, mean radicle length, plant survival, plant height and dry weight were determined by dividing the totals of the parameter measured by the total number of seeds germinated or plants survived. If germination failed to occur in a replicate, then the replicate mean, or zero value, was not included in the average. In these cases, the detrimental effect would be expressed as germination and survival inhibition. Zeros were included in the averages for seed germination and seedling survival data analysis.

Findings:

Test concentrations do not appear to have been verified analytically.

Seed Germination: The monocots were generally less sensitive than the dicots in the seed germination study. Onion, field corn, wheat and oat showed no significant differences between non-treated controls and any treatment rate. For the dicots, a detrimental treatment effect was observed and NOELs determined for all crops except soybean and tomato where seed germination was not affected at the highest treatment rate.

Radicle length was a more sensitive indicator for adverse effects from 2,4-D BEE exposure, and NOELs were generated for all crops.

Visual observations: Phytotoxic effects were noted in all of the crops within the rate range tested, with the exception of field corn and oat. Although wheat showed slight injury at 7 days after treatment, it fully recovered within 28 days. Phytotoxic symptoms were observed within 7 days and were similar throughout the duration of the study. By 28 days after treatment, radish and tomato showed the most severe injury with the most common symptoms of epinasty and stunting at the two highest rates tested of 560 and 280 g ae/ha. Soybean, sunflower and onion were moderate in phytotoxic response. Cucumber and carrot showed some injury, but only at the highest rate tested, 560 g ae/ha.

Seedling Emergence:

Emergence and Survival

2,4-D BEE had little effect on seedling emergence or plant survival. There were no substantial changes in plant numbers at 14 days (emergence) and 28 days (survival), consequently the 28 day data were used to compute the NOEL, ED25 and ED50 values. Radish and onion were the only crops to show a significant seedling emergence and survival response. These two plants were the only ones analysed by regression analysis.

Plant Height

Statistical NOECs were determined for soybean, radish, sunflower and tomato, which showed significant plant height reductions at the tested rates. The remaining crops showed no statistical differences compared to non-treatment controls. With the exception of field corn and oats, all crops showed phytotoxic response (epinasty); however, there were no effects on plant height at 28 days after treatment.

Plant Dry-Weight

Among the monocots, wheat was the most tolerant species. Field corn and oats showed significant treatment effects at the higher rates. Interestingly, dry weight was the only parameter affected for field corn and oats. There were no other treatment effects observed in these two crops. Among the dicots, only tomato and radish showed significant treatment effects.

For dry weight, statistical analysis was based on individual plant weights rather than replicate values. The overall biomass per treatment can differ due to plant survival differences and when plant stands are reduced, some plants compensate with more abundant growth. For example, there was around a 42% reduction in carrot plant survival at 560 g ae/ha, but this resulted in only a 10% reduction in plant dry weight on a per plant basis.

Statistical Results

The results of the statistical analysis are shown in the following tables

Table A2.57: Effects of 2,4-D BEE on Germination and Radicle Length of ten plant species (results in g ae/ha)

		Seed Germination			Radicle Length		
		NOEL	ED25	ED50	NOEL	ED25	ED50
Monocots	Onion	>63	>63	>63	0.25	0.33	34.4
	Field corn	>63	>63	>63	1	16.8	41.6
	Oat	>63	>63	>63	1	12	29.6
	Wheat	>63	>63	>63	0.25	ND	21.6
Dicots	Soybean	>63	>63	>63	1	0.8	21.6
	Radish	1	16.8	38.4	0.016	ND	1.6
	Cucumber	16	56.8	>63	0.25	ND	18.4
	Sunflower	1	4	6.4	0.25	0.8	1.6
	Tomato	>63	>63	>63	1	17.6	36
	Carrot	1	10.4	23.2	0.25	4.8	16

Table A2.58: Effects of 2,4-D BEE on Seedling Emergence End-points of ten plant species (results in g ae/ha) -

		Emergence/Survival			Dry Weight			Plant Height		
		NOEL	EC25	EC50	NOEL	EC25	EC50	NOEL	EC25	EC50
Monocots	Onion	280	434	547	>560	ND	>560	>560	ND	>560
	Field corn	>560	>560	>560	280	>560	>560	>560	>560	>560
	Oat	>560	>560	>560	280	>560	>560	>560	>560	>560
	Wheat	>560	>560	>560	>560	>560	>560	>560	>560	>560
Dicots	Soybean	>560	>560	>560	>560	>560	>560	140	>560	>560
	Radish	140	237	405	280	251	487	35	301	>560
	Cucumber	>560	>560	>560	>560	>560	>560	>560	>560	>560
	Sunflower	>560	>560	>560	>560	>560	>560	280	>560	>560
	Tomato	>560	>560	>560	35	57	510	280	>560	>560
	Carrot	>560	>560	>560	>560	>560	>560	>560	510	>560

Conclusions:

Of all the parameters measured, radicle length was the most sensitive indicator of 2,4-D BEE injury. While these effects were more prominent because the chemical was in direct continuous contact with the germinating seedling, the implications of these data will be more fully analysed in the risk assessment.

Fewer effects were found when 2,4-D BEE was applied to the soil surface because of the potential for the chemical to dissipate from the system prior to being taken up by plants.

Test Material: **2,4-D 2-EHE**

Report: Backus and Crosby, 1992b

Guidelines: US EPA Guideline 123-1 (b) Tier II

GLP: No – QA statement provided.

Test system:

Tier II vegetative vigour studies were conducted to evaluate non-target phytotoxicity of 2,4-D 2-EHE to 6 dicotyledonous plant species and 4 monocotyledonous plant species. Seeds were separately planted in 9 cm square pots containing a commercial soilless growing medium. For corn, cucumber, soybean and tomato, several seeds per pot per species were planted to a depth of approximately 1.0-1.5 cm, depending upon the size of the seed. For buckwheat, mustard, oats, onion, radish and sorghum, seed was sprinkled on the medium surface in the pot and covered with around 0.5-1.0 cm

of additional medium. Test material application occurred on a uniform population and seedlings were thinned at emergence to avoid crowding.

The plants were grown according to an empirically derived schedule that ensured all plants were growing vigorously at the time of application. Plant species were within 7-21 days from planting at application. Each treatment in the definitive study included 3 replicates. Each replicate contained 5 pots of corn, cucumber, soybean and tomato. Each of these five pots contained one plant. Each replicate consisted of one pot each of a representative population of buckwheat, mustard, oats, onion, radish and sorghum. There were three solvent control replicates (acetone only) as well as three untreated control replicates.

Application rates were determined based on preliminary testing and typical application rates, and were set to be 1080, 540, 270, 135, 67.5, 33.6, 16.8 and 8.4 g ae/ha. Analysis was performed on samples of some of these rates.

Fourteen days after application, visual observations for survival and phytotoxicity were recorded. Fresh weight measurements were taken at day 14 and apart from visual observations, this was the only end-point assessed analytically.

The NOEL was estimated using a one-way ANOVA model. The EC25 and EC50 values were calculated using regression analysis.

Findings:

Test concentrations in spray mixtures ranged from 97.4-100% of nominal values.

Visual observation: After 14 days from treatment, stunting, distortion and necrosis were observed on buckwheat, cucumber, mustard, onion, radish, soybean and tomato at the highest rate tested. Tip burn and stunting were observed in oats and sorghum respectively at this concentration. Since oats in the solvent control on this date were observed to exhibit burning and necrosis, the symptoms were believed to be a solvent effect and not treatment-related. Corn appeared normal, but was falling over. Stunting was observed on oats and sorghum at 540 g ae/ha, and corn again appeared to be falling over. All other species continued to exhibit the same symptoms as observed at the high rate. The visual NOEL for corn and oats appeared to be 270 g ae/ha. At this rate, buckwheat, cucumber, onion and tomato evidenced stunting and distortion while mustard, radish and soybean also evidenced necrosis. The visual NOEL for sorghum appeared to be 135 g ae/ha. For the most part, symptoms of stunting and distortion appeared similar to those observed at the previous rate.

At 67.5 g ae/ha, symptoms were somewhat less pronounced. Stunting and distortion were observed on buckwheat, cucumber and tomato while only stunting was observed on mustard, onion, radish and soybean. At 33.6 g ae/ha buckwheat no longer exhibited distortion, however, all other species exhibited responses similar to those observed at the 67.5 g ae/ha level. The same was true of the 16.8 g ae/ha level. At the lowest rate tested of 8.4 g ae/ha, buckwheat and tomato appeared stunted. Cucumber and soybean evidenced stunting and distortion and all other species exhibited no effects.

Statistical results:

The results of the statistical analysis are shown in the following table.

Table A2.59: Effects of 2,4-D 2-EHE on vegetative vigour of ten plant species (results in g ae/ha)

		Fresh Weight		
		NOEL	EC25	EC50
Monocots	Onion	270*	≥270*	290
	Corn	≥1080	≥1080	≥1080
	Oat	≥1080	≥1080	≥1080
	Sorghum	67.5	244	850
Dicots	Soybean	8.4	22.4	740
	Radish	16.9	33.6	135
	Cucumber	16.8	215	874
	Buckwheat	16.9	23.5[1]	89.7[2]
	Tomato	6.72*	11.2[3]	65.0[4]
	Mustard	33.6	33.6-67.5	67.5-135

* - See discussion below
Reported confidence limits were (g ae/ha): **1)** 15.7-29.1; **2)** 69.5-115.4; **3)** 6.72-16.9; and **4)** 48.2-85.2

Statistically, onion was the most sensitive monocot. While a linear regression was generated, the data for this species were variable. Significant reductions were computed by ANOVA at 540 and 1080 g ae/ha. The 270 g ae/ha level elicited a 52.5% weight reduction due to data variability, however, this was not significantly different from the control. The regression derived values were therefore overridden.

The most sensitive dicot was tomato, exhibiting a linear dose response. The statistically generated NOEL value was ≤8.4 g ae/ha, the lowest level tested. To yield a defined NOEL, the value determined in the preliminary study, 6.72 g ae/ha, was used.

Although on gross observations cucumber exhibited phytotoxicity to the test material, this species was the least sensitive dicot in terms of statistical analysis of fresh weight.

Conclusions:

Dicots were much more sensitive to monocots when exposed to 2,4-D 2-EHE in a vegetative vigour study. Tomatoes were the most sensitive plants with a NOEL of 6.72 g ae/ha fresh weight, and an EC25 of 11.2 g ae/ha.

Test Material: **2,4-D BEE**

Report: Harnish, 1993

Guidelines: US EPA Guideline 123-1 (b) Tier II

GLP: No – QA statement provided.

Test system:

Tier II vegetative vigour studies were conducted to evaluate non-target phytotoxicity of 2,4-D BEE to 6 dicotyledonous plant species and 4 monocotyledonous plant species. At least 10 seeds of each test species were separately planted in 15 cm round pots containing pasteurised sandy soil. Small seeded plants (carrot, onion, radish and tomato) were planted to 0.5-1 cm. Large seeded plants (corn, wheat, oat, cucumber, sunflower and soybean) were planted to 1-1.5 cm. Four replicate pots for each treatment were prepared for each species.

Five dosage rates were tested. Corn, wheat and oats were exposed to 560, 280, 140, 70 and 35 g ae/ha (two fold dilution for each successive treatment rate). Crops vary widely in their response to 2,4-D BEE and other phenoxy herbicides with broadleaf

plants being more sensitive than grass crops. To provide a rate range that would encompass a NOEL for all crops, carrots, cucumber, sunflower, onion, radish, soybean and tomatoes were exposed to a four fold dilution series with treatment rates of 560, 140, 35, 8.75 and 2.19 g ae/ha. In addition, a dilute solvent control and untreated control was maintained.

Test treatments were applied as a foliar spray using a single nozzle, hand held, CO_2 pressurized spray calibrated prior to application.

Visual observations for survival and phytotoxicity were evaluated at 7, 14 and 21 days after treatment. The 21-d data were used to determine the NOELs. Seedling number and plant height were recorded at –7, 0, 14 and 21 days. Mean plant height was calculated for each treatment. Dry weight measurements were taken at day 21 with the above ground portion of the plants used. Replicate means were used for statistical analysis.

The NOEL was estimated using a one-way ANOVA model. The EC25 and EC50 values were calculated using regression analysis.

Findings:

Test concentrations were not verified analytically.

Visual observation: No phytotoxic effects were observed in the solvent or non-treated controls.

Monocots: Field corn was the most tolerant crop with no phytotoxic effects at any of the rates or evaluation periods. Although wheat and oats showed slight epinasty within 7 days of treatment at the highest rate tested, by day 21 the plants had recovered. Onion was the most sensitive monocot and exhibited severe epinasty and stunting. By 21 days, some plant death occurred at the highest rate.

Dicots: Cucumber and soybean were the most tolerant dicots. At 560 g ae/ha, moderate epinasty and slight yellowing of plants within 7 days was observed and continued until day 21 for both crops. The remaining dicots were much more sensitive. The two highest rates (560 and 140 g ae/ha) caused moderate to severe epinasty and leaf yellowing with 7 days. By 21 days, the 560 g ae/ha caused death in carrot, radish, tomato and sunflowers. The tomato species showed phytotoxic symptoms (epinasty, chlorosis and stem thickening) at rates as low as 8.75 g ae/ha.

Statistical results:

The results of the statistical analysis are shown in the following table.

Table A2.60: Effects of 2,4-D BEE on vegetative vigour of ten plant species (results in g ae/ha)

		Plant Height			Dry Weight		
		NOEL	EC25	EC50	NOEL	EC25	EC50
Monocots	Onion	35	337	>560	35	212	383
	Field corn	>560	>560	>560	>560	447	>560
	Oat	280	>560	>560	>560	>560	>560
	Wheat	>560	>560	>560	>560	>560	>560
Dicots	Soybean	35	268	520	35	115	349
	Radish	35	50	77	35	24	66
	Cucumber	140	>560	>560	35	>560	>560
	Sunflower	35	74	141	35	50	90
	Tomato	35	61	121	35	33	76
	Carrot	35	92	182	35	84	138

These results indicate that terrestrial plants are not as sensitive to 2,4-D BEE as they are to 2,4-D EHE (see results in Table A2.59 above). However, the authors of this study note that the decreased activity of this ester can be explained by the environmental conditions prevailing throughout the study. The air temperature at the time of spray application was >90^{o}F which is conducive to evaporation of the spray droplets. Therefore the decreased activity is likely due to the volatility of the ester.

Conclusions:

Dicots were much more sensitive to monocots when exposed to 2,4-D BEE in a vegetative vigour study. Radish were the most sensitive plants with NOELs of 35 g ae/ha for both plant height and dry weight, and EC25 values of height and weight of 50 and 24 g ae/ha respectively.

In addition to the above studies, test reports not provided to the APVMA were assessed by the US EPA for toxicity of 2,4-D IPE to various monocots and dicots in both seedling emergence and vegetative vigour studies. The results of these tests as reported in US EPA, 2005 are summarised as follows:

Table A2.60: Additional Seedling Emergence Results as Reported in US EPA, 2005

Species	NOEL – g ae/ha	EC25– g ae/ha	Endpoint affected
2,4-D IPE			
Monocot - Onion	6.3	11.2	Shoot length
Monocot - Oats	104		Shoot length
Dicot - Root Crop (Turnip)	6.6		Shoot length
Dicot - Soybean	113		
Dicot -Cabbage	1.0	27.3	Emergence
Dicot - Lettuce	0.5	0.9	Shoot length
Dicot - Cucumber	26.4		Shoot length
Dicot - Tomato	12.2	22.6	Shoot length

Table A2.61: Additional Vegetative Vigour Results as Reported in US EPA, 2005.

Species	NOEL – g ae/ha	EC25– g ae/ha	Endpoint affected
2,4-D IPE			
Monocot - Corn	28.2	226	Shoot weight
Dicot - Root Crop - Radish	4.0	4.7	Root weight
Dicot - Cabbage	7.5	10.4	Root weight
Dicot - Cucumber	64.0	145	Shoot weight
Dicot - Tomato	7.5	19.8	Root weight
Dicot - Root Crop (Turnip)	5.6	17.9	Dry weight
Dicot - Soybean	113		
Dicot -Cabbage	2.8	12.2	Dry weight
Dicot - Lettuce	6.9	1.4	Dry weight
Dicot - Tomato	29.2	45.2	Dry weight

Lettuce was very sensitive to this ester in both the seedling emergence and vegetative vigour study being the most sensitive dicot in both.

In addition, some old data are reported for terrestrial plant toxicity from exposure to 2,4-D Ethyl ester in the ECOTOX database. These data are all reported from the 1960's and the results as presented are difficult to interpret so have not been reported here.

Conclusions for Terrestrial Toxicity

2,4-D EHE was, at worst, slightly toxic to bees through either contact or oral exposure routes with LD50 values of >64.3 and >62.9 µg/bee respectively. A non-standard test using 2,4-D EE to an Indian worker bee show this chemical to have an LD50 of 0.013% v/v, but this value can not readily be translated to a rate per bee or a rate per hectare. No other bee toxicity data were available for any other esters. 2,4-D EHE was harmless to the predatory mite up to the highest rate tested of 564 g ae/ha. However, at this rate it was harmful to the parasitic wasp, causing 100% mortality of adults. It was harmless to this species at the next lowest rate tested of 28 g ae/ha. No other ester form of 2,4-D was tested on any other beneficial insect. Additionally, no ester toxicity data to earthworms or soil micro-organisms were available.

Data available for non-target terrestrial plants show vegetation to be highly susceptible to damage from 2,4-D esters. Generally, monocots were not as sensitive as dicots. Germination studies showed in preliminary testing (2,4-D EHE) and definitive testing (2,4-D BEE) that radicle length was the most sensitive indicator. Results of vegetative vigour studies, particularly for 2,4-D EHE, indicate 2,4-D esters will have significant impacts on actively growing plants, often at relatively low rates (<30 g ae/ha).