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 III- 2,4-D Amine Salts

APRIL 2006

Australian Pesticides &

Veterinary Medicines Authority

Canberra

Australia
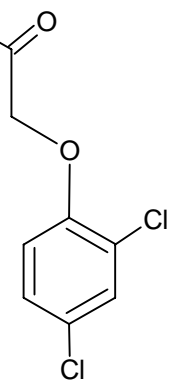
APPENDIX III – Technical Report for Amine Salts of 2,4-D

Table of Contents:

Identity, Physical and Chemical Properties, 2,4-D Dimethylamine Salt (US EPA, 2005)

	Dimethylamine Salt
Common name (ISO)	2,4-D DMA
Chemical name	2,4-D Dimethylamine salt
CAS No	2008-39-1
Molecular formula	$C_{10}H_{13}Cl_2NO_3$
Molecular mass	266.13
Structural formula	
Relative density	pure a.s.: 1.1502 technical a.s.: 1.1527
Vapour pressure	Dissociates rapidly to 2,4-D acid
Henry's law constant	Not reported - Dissociates rapidly to 2,4-D acid
Solubility in water[1]	pH 5 320632 ± 3645 mg/L @ 25^0C pH 7 729397 ± 86400 mg/L @ 25^0C pH 9 663755 ± 94647 mg/L @ 25^0C
Partition co-efficient (log Kow)	Not reported - Dissociates rapidly to 2,4-D acid

1)   Hopkins, 1987a.

With respect to amine salts of 2,4-D, all data received by the APVMA were for 2,4-D DMA as described above. Three other amine salts are also used in formulations registered in Australia include 2,4-D isopropylamine (IPA), 2,4-D triisopropanolamine (TIPA) and 2,4-D diethanolamine (DEA). The only other data that seemed to be reviewed by the US EPA in their reregistration assessment of 2,4-D were dissociation studies for all three amine salts. It is unclear why these data were not also made available to the APVMA. However, data assessed by the US EPA with respect to these amine salts will be reported in the overview report.
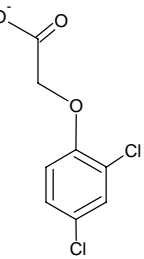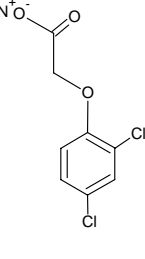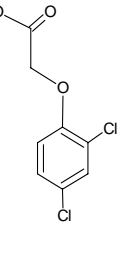
For the US EPA assessment, the registrant submitted bridging data on the dissociation of 2,4-D amine salts, specifically for 2,4-D DMA, 2,4-D IPA, 2,4-D and 2,4-D TIPA (APVMA received only the study for 2,4-D DMA). Based on these data, the 2,4-D amine salts have been shown to dissociate rapidly in water with the bridging data indicating under most environmental conditions 2,4-D amine will rapidly form 2,4-D acid.

The weight of evidence from the open-literature and registrant sponsored data reviewed subsequent to establishment of the bridging strategy indicates that 2,4-D amine salts are not persistent under most environmental conditions including those associated with most sustainable agricultural conditions. 2,4-D amine salt

dissociation is expected to be instantaneous (<3 minutes) under most environmental conditions (US EPA, 2005).

Following are the identity and physico chemical properties of the three amine salts as described by the US EPA, all of which are registered in Australia.

Common name:	2,4-D IPA	2,4-D TIPA	2,4-D DEA
Chemical name:	isopropylamine 2,4-dichlorophenoxyacetate	triisopropanolamine 2,4-dichlorophenoxyacetate	Diethanolamine 2,4-dichlorophenoxyacetate
Molecular formula:	$C_{11}H_{15}Cl_2NO_3$	$C_{17}H_{27}Cl_2NO_6$	$C_{12}H_{17}Cl_2NO_5$
CAS Number:	5742-17-6	32341-80-3	5742-19-8
Molecular weight:	280.04	412.31	326.18
Structure:	$[(CH_3)_2]HCHN]^+O^-$	$(H_3CCHOHCH_2)_3HN^+O^-$	$[(HO(H_2C)_2)_2H_2N]^+ \text{-}O$
Vapor pressure (20°C):	Decomposed to acid (-3.9 to 24°C)[1]	$<1 \times 10^{-7}$ mmHg @ 14 – 28°C[2]	1.33×10^{-5} pa @ 25C[3]
Henry's Law:	Not reported. Dissociates rapidly to acid	Not reported. Dissociates rapidly to acid	5.38×10^{-9} pa m³/mol
Solubility (25°C):	373 g/L @ 20°C		806 g/L
Log K_{ow}:	Not reported. Dissociates rapidly to acid	Not reported. Dissociates rapidly to acid	-1.65 @ 25 C[4]

1) MRID 41431101 – Study not provided to APVMA
2) MRID 41431201– Study not provided to APVMA
3) MRID 42857206– Study not provided to APVMA
4) MRID 42857207– Study not provided to APVMA

Hydrolysis

No data were provided for this end-point.

Photodegradation in Water

No data were provided for this end-point.

Photodegradation in Soil

No data were provided for this end-point.

Degradation in Soil and Water

Soils – Aerobic

No data were provided for this end-point.

Soils – Anaerobic

No data were provided for this endpoint.

Water – Aerobic

No data were provided for this end-point.

Water – Anaerobic

No data were provided for this end-point.

Mobility

Adsorption/Desorption

No data were provided for this end-point.

Leaching Potential

Column Leaching Studies

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

Aged Column Leaching Studies

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

Lysimeter/Field Leaching Studies

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

Report: Burgener, 1993

Guidelines: BBA Teil IV, 4-3

GLP: Yes

Test System

Undisturbed soil monoliths were excavated (surface area 1 m^2, depth, 1.2 m), from a site with the following soil characteristics:

Table A3.1: Soil characteristics in the top 30 cm

Texture	% Sand	% Silt	% Clay	% OM	Biomass (mgC/100 g)	pH	Field Capacity	Density (g/cm^3)
Sandy	68.3	24.5	7.2	1.5	20.4	5.7	20.34	1.50

The lysimeters were embedded into the ground at an outdoor experimental station to soil level and each was surrounded by a small field plot of about 3 m diameter to simulate actual field conditions. For the study, 2 treated lysimeters and one control lysimeter were used.

The formulation used for application was a DMA suspension concentrate containing 500 g ai/L. The application rate was 1.5 L/ha, or 750 g ai/ha. In acid equivalent terms, this corresponds to around 625 g ae/ha. Precautions were taken to protect the surrounding areas of the lysimeters during application by enclosing them in polyethylene foils. Following application, the inner plastic foil was removed and the radioactivity remaining on the foil and in the application vessel was determined to assess the magnitude of pesticide drift and thus obtain the actual amount of pesticide applied to the lysimeters.

Air temperature, soil temperature in 10 cm and 30 cm depth, air humidity, wind speed and precipitation were recorded continuously. In drier periods, additional rainfall was applied to the lysimeters. Leachate was sampled depending on rainfall and other climatic conditions. The collection vessels were regularly checked and when there was sufficient volume the entire sample was pumped out for analysis of radioactivity by LSC. After the first vegetation period, 110 days after application, radioactivity in the topsoil (0-10 cm) was analysed. Then, at the end of the trial (around 26 months), the lysimeters were excavated and divided into 10 cm horizontal layers.

In the leachates, $^{14}CO_2$ and other volatiles were determined through acidification of leachate samples with the gaseous components being stripped by a constant stream of nitrogen. This was bubbled through three gas-washing bottles to trap the volatiles. Where leachates contained more than 0.05 ppb parent equivalents (after stripping volatiles), they were enriched for analysis. TLC and HPLC analyses were performed on the extracts.

Radioactivity taken up by plants during the various vegetation periods were analysed by homogenising plant samples and combusting. Liberated $^{14}CO_2$ was absorbed in a solvent solution then mixed with a scintillation cocktail and radioactivity measured.

The 110 day soil samples were extracted as follows: a sample of thoroughly mixed moist soil was combined with a solvent and shaken for 30 minutes after which it was centrifuged and the supernatant submitted to LSC analysis. This extraction step was performed three times each with two solvent systems. The first extracts of each solvent were combined and the radioactivity characterised by TLC.

Following sectioning of the lysimeters at the end of the study, samples of each soil layer were analysed for total radioactivity by combustion. Extraction was largely as above for the 110 day samples, except only a single acetonitrile/water/hydrochloric acid solvent system was used instead of the two solvent systems. Following the soil extractions at room temperature, an overnight extraction with Soxhlet, using the same solvent was carried out. The extracts were concentrated and used for TLC analysis.

Findings:

Application rates of 92.6% and 94% were achieved on lysimeters 1 and 2 respectively. During the first year, the natural precipitation amounted to 1003 mm (the required amount for this German study was 800 mm as this is considered a realistic German average). Starting two days after application, a series of exceptionally heavy rainfall events were recorded and the monthly amount falling in the month of application was 225 mm (compared to the long term average of 112 mm for that month). During the first year, the amount of leachate was 484 and 490 L in lysimeters 1 and 2 respectively. In total, 958 and 994 L leachate were collected in lysimeters 1 and 2 respectively, corresponding to around 48% and 50% respectively of total precipitation/irrigation.

Mass Balance

Table A3.2: Radioactivity Balance During the Two Growing Seasons:

Lysimeter (%)	Leachate (not with $^{14}CO_2$)	Leachate dissolved $^{14}CO_2$.	Soil layers	Plants, total	$^{14}CO_2$ and other volatiles – atmosphere[a]
Lysimeter 1	0.10	0.10	20.95	2.84	76.01
Lysimeter 2	0.19	0.06	16.82	1.67	81.26

a) Determined by difference (that is, 100% less all other findings).

Besides CO_2, no other volatile substances were detected in the leachates.

Transformation of parent compound

Leachate: 2,4-D as well as the metabolites 2-CP, 4-CP and 2,4-DCP were not detected in any of the analysed leachates. In both lysimeters, up to three unknown radioactive fractions were detected and remained unidentified. Besides minor amounts of two metabolites, one major radioactive fraction was present in each leachate sample worked up. This amounted to 0.043% and 0.087% in lysimeters 1 and 2 respectively.

<u>Soil:</u> In the soil, the total recovered radioactivity in the soil profile amounted to 20.95% (11.44% in the topmost layer) and 16.82% (8.79% in the topmost layer) for lysimeters 1 and 2 respectively. In both, only about 0.1% AR was found in the soil profile below 57 cm. The nature of the extractable radioactivity of the soil originated from 3 unknown radioactive fractions. The pattern and amount of metabolites of the soil extracts of both lysimeters compared well and were nearly identical. Fractions of the active ingredient applied were found as deep as 17 cm and amounted to a total of 0.26% and 0.29% AR in the topsoil of lysimeters 1 and 2 respectively. The known metabolites of 2-CP, 4-CP and 2,4-DCP were not detectable in any of the six soil layers extracted to a soil depth of 57 cm.

Conclusion

The number and amount of metabolites found in the leachate and soil samples compared well in both lysimeters. The results obtained indicated that ^{14}C-2,4-D and its metabolites are not mobile in the sandy soil tested. The nature of the leached radioactivity completely originated from very polar unidentified residues.

Fate and Behaviour in Air

Modelled data:

For three of the amine salts, the US EPA has noted that Henry's Law Constants were not reported due to rapid dissociation to form 2,4-D acid. However, for 2,4-D DEA, a Henry's Law Constant of 5.8×10^{-9} Pa m^3/mol is reported. This value is indicative of only very slight volatility from water (Mensink *et al*, 1995).

No experimental data for degradation or volatility in the atmosphere were provided and given the rapid conversion to the acid, this is acceptable. However, the former has been considered through modelling. For the four 2,4-D amine salts (DMA, IPA, TIPA and DEA), 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:

$$t_{\frac{1}{2}} = \ln2/k' = \ln2/k_{OH} \times [\text{OH radicals}]$$

The diurnally and seasonally averaged concentration of tropospheric hydroxyl radicals used by the AOP program is 1.5×10^6 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 (hours)
2,4-D DMA	CN(C)(H)(H)OC(=O)COc1c(Cl)cc(Cl)cc1	6.1290	41.88
2,4-D IPA[2]	OC(=O)COc1c(Cl)cc(Cl)cc1	6.6262	38.74
2,4-D TIPA[2]	OC(=O)COc1c(Cl)cc(Cl)cc1	6.6262	38.74
2,4-D DEA[2]	OC(=O)COc1c(Cl)cc(Cl)cc1	6.6262	38.74

1)   Rate constant, K_{OH} ($\times 10^{-12}$ cm^3/molecule/second)
2)   Calculated by the program as for 2,4-D Acid.

Field Dissipation Studies

Forestry

Test Material:	**2,4-D DMA**
Report:	Barney, 1995f.
Guidelines:	US-EPA Subdivision N; 164-3
GLP:	Yes

Test System

2,4-D DMA has been shown to dissociate 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 DMA) 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 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 Parkdale, Oregon and involved one treated and one non-treated site. The treated field contained 10 sampling areas (5 for exposed soil cores and 5 for foliage, leaf litter and protected soil cores) while the UTC contained three sub-plots.. The soil characteristics of the field were as follows:

Table A3.3: Soil and sediment characteristics

Sampling depth	Texture	% Sand	% Silt	% Clay	% OM	CEC (meq/100 g)	pH	% MC
Soil								
0-15 cm	Loam	50.4	38.0	11.6	9.50	18.07	6.6	34.61
15-30 cm	Sandy loam	58.4	34.0	7.6	11.14	19.40	6.3	37.90
30-45 cm	Sandy loam	54.4	36.0	9.6	4.20	11.56	6.4	30.23
Sediment (in the 0-5 cm layer)								
Treated	Loam	4.04	38.0	21.6	5.51	12.46	6.3	38.01
Untreated control	Sandy Loam	72.4	18.0	9.6	1.86	7.70	6.8	21.86

The soil had a bulk density of 1.01 g/cm^3 in the top 15 cm layer. The sediment in the treated plot had a bulk density of 0.99 g/cm^3 while the untreated plot sediment had a bulk density of 1.22 g/cm^3.

The experimental site was prepared by creating the exposed soil site with a crawler by removing brush 8 days prior to test substance application. The chemical was applied in the formulated product, Amine 400 2,4-D Weed Killer using a helicopter. At application the boom was around 6.1 m above the canopy. 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 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, 14, 30, 59, 90, 120, 181, 361 (protected soil), 398 (exposed soil) and 421 (protected soil). 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.

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Foliage (Giant Chinquapin – *Castanopsis chrysophylla* (Dougl.)) 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 90 and 181 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:

Natural precipitation for the trial period amounted to 84% of the 30 year historical precipitation average. Application was undertaken on 15 June 1993. The rainfall averages for June, July and August 1993 were 280%, 380% and 15% of the 30 year averages.

Meteorological data provided in the study report showed 0.06 inches (1.5 mm) of rain falling on the day of application. The next rain periods did not occur until days 7 and 8 after application where 1.3 mm and 5.3 mm fell respectively.

Based on the 15 application monitoring pads within the treated area, the average actual application rate was 3.45 kg ae/ha (77% of the target rate).

Table A3.4: Residues of 2,4-D, 2,4-DCP and 2,4-DCA (ppm) in four tested media.

Analyte[1]	0	1	3	7	14	30	59	90	120	181
Exposed Soil										
2,4-D	2.486	1.178[2]	0.357	0.883[3]	0.18[4]	0.359	0.167	0.086	0.218[5]	0.025
2,4-DCP	-	0.013	-	0.031	0.008	0.023	0.011	0.004	0.013	0.017
2,4-DCA	-	-	-	-	-	-	-	-	-	0.021
Protected Soil										
2,4-D	0.685	1.015	0.398	0.234[6]	0.276	0.095	0.027	0.166	0.027	0.019
2,4-DCP	-	0.021	0.017	-	0.02	0.014	0.007	0.012	-	0.01
2,4-DCA	-	-	-	-	0.018	0.012	-	-	0.003	0.02
Foliage (ppm dry foliage)										
2,4-D	172.3	110.5	120.6	29.7	51.34	23.59	14.50	4.73	1.358	0.828
2,4-DCP	0.110	0.485	0.721	0.922	1.05	0.716	0.446	0.273	0.225	0.103
2,4-DCA	0.007	0.026	0.087	0.048	0.035	0.024	0.016	-	-	-
Leaf Litter (ppm dry leaf litter)										
2,4-D	26.61	35.14	24.73	43.31	9.471	7.41	8.87	8.03	5.14	1.71
2,4-DCP	0.585	0.912	0.932	2.79	0.842	1.03	0.684	0.753	0.457	0.344
2,4-DCA	-	-	0.109	0.105	0.066	0.152	0.085	0.103	0.136	0.124

1 LOQ = 0.01 ppm for soil and leaf litter; 0.1 ppm for foliage.
2 2,4-D found at an average (3 replicates) of 0.04 ppm in the 15-30 cm layer.
3 2,4-D found at an average (3 replicates) of 0.038 ppm in the 15-30 cm layer.
4 2,4-D found at an average (3 replicates) of 0.004 ppm in the 15-30 cm layer.
5 2,4-D found in one replicate at 0.065 ppm in the 30-45 cm layer (not detected in the other replicates).
6 2,4-D found at an average (3 replicates) of 0.013 ppm in the 15-30 cm layer.

In exposed soil, 2,4-D was last detected at 181 DAA. No residues were found above the LOQ at the 398 day sampling interval. Residues of 2,4-D were mainly retained in the top 15 cm of soil with some detections found in the 15-30 cm layer and one

sample showing residues at day 121 in the 30-45 cm layer. Residues of 2,4-DCA were only found above the LOQ once at 181 DAA.

In protected soil, residues were almost exclusively retained in the top 15 cm with the exception of one replicate in the 15-30 cm layer at 7 DAA showing 2,4-D residues at a concentration of 0.038 ppm (average of 0.013 ppm for three replicates). In protected soil, this compound was still detected in one replicate at 0.012 ppm 360 DAA but was not detected at 421 DAA.

In foliage, 2,4-D and 2,4-DCP were still detected at concentrations of 0.220 and 0.049 ppm dry foliage respectively at 360 DAA while all three analytes were found at 360 DAA in leaf litter at concentrations of 0.711, 0.167 and 0.039 ppm dry leaf litter for 2,4-D, 2,4-DCP and 2,4-DCA respectively.

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 A3.5 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 A3.5: 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	-0.0179	38.7[1]	0.6794
Protected soil	2,4-D	-0.0137	80.8[2]	0.7677
Foliage	2,4-D	-0.0185	37.84[2]	0.8429
	2,4-DCP	-0.0086	80.8[3]	0.9051
Leaf litter	2,4-D	-0.0106	65.7[2]	0.8411
	2,4-DCP	-0.0062	111.3[4]	0.7855

1	Calculated from day 0 – 181
2	Calculated from day 0 – 360
3	Calculated from day 14 - 360
4	Calculated from day 7 – 360

Conclusion:

2,4-D showed the least persistence in exposed soil and foliage where it dissipated with a half-life of around 5.5 weeks. Where conditions were more sheltered 2,4-D was more persistent with half-lives in protected soil and leaf litter of 11.5 and 9.4 weeks respectively. All analytes appeared more persistent in leaf litter and were found at 360 days after application. Patterns of formation and decline for 2,4-DCP could be detected in both foliage and leaf litter where it demonstrated half-lives in the order of 11.5 and 16 weeks respectively.

Pasture

Two field studies were provided for application of 2,4-D DMA to pasture applied as WEEDAR Brand 64 Broadleaf Herbicide (46.8% DMA; 38.9% acid equivalent – ae) 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 DMA**	**2,4-D DMA**
Report:	Barney, 1995g.	Hatfield, 1995i
Guidelines:	US-EPA Subdivision N; 164-1	US-EPA Subdivision N; 164-1
GLP:	Yes	
Test System		
Location:	Tulare County, California.	Pattison, Texas.
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 top 15 cm.

Location	Texture	% Sand	% Silt	% Clay	% OM	CEC (meq/100 g)	pH	% MC
California	Sandy loam	62	32	6	3.3	12.1	7.2	17.8
Texas	Sandy loam	61.6	29.6	8.8	1.46	3.29	6.1	11.66

The bulk density of the Californian soil was 0.95 g/cm^3 while that for the Texas soil was 1.56 g/cm^3

Experimental treatments:	Two applications were made. The first when weeds were immature and the second between 26 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 ground or crop surface. The target application rate was 2.25 kg ai/ha (Barney, 1995g) and 2.5 kg ai/ha (Hatfield, 1995i) 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 one occasion in Hatfield, 1995i.
Sampling	Sampling was undertaken according to the following regime:
	California: soil samples were collected from the untreated and treated plots at –1, 0, 1, 3, 7, 14 and 29 (one day prior to the second application) DAT for the first application, and 0, 1, 3, 7, 14, 30, 60, 90, 120, 180, 270 and 300 DAT for the second application.
	Texas: soil samples were collected from the untreated and treated plots at –1, 0, 1, 3, 7 and 25 (one day prior to the second application) DAT for the first application, and 0, 1, 3, 7, 15, 30, 64, 94, 120 and 175 DAT for the second application.
	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. (Only the top 15 cm was sampled on the days of application).

Analysis   Soil residue data were generated for 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. The first contained 2,4-DCP and 2,4-DCA. The second fraction, containing 2,4-D, was concentrated and methylated to form the methyl ester (2,4-D ME), then partitioned (petroleum ether/hexane). The two fractions were then combined into a single solution for chromatographic analysis.

Results and Discussion

Application   Recoveries achieved on analysis of the application monitors at the
verification: Californian trial were 79% (application 1) and 83% (application 2). These recoveries were the mean of 9 separate monitors.

Recoveries achieved on analysis of the application monitors at the Texas trial were 121% (application 1) and 83% (application 2). These recoveries were the mean of 15 separate monitors.

Findings:

Table A3.6: Residue Formation and Decline in the Californian Trial (ppm).

Time	Cumulative	2,4-D	2,4-DCP	2,4-DCA
0 DA1A	0	0.417	-	-
1 DA1A	1	0.348	-	-
3 DA1A	3	0.571	-	-
7 DA1A	7	0.311	0.008	0.019
14 DA1A	14	0.047	-	0.016
-1 DA2A	29	0.018	-	-
0 DA2A	30	0.336	-	-
1 DA2A	31	0.560	-	-
3 DA2A	33	0.395	-	-
7 DA2A	37	0.131	-	0.007
14 DA2A	44	0.146	-	-
30 DA2A	60	0.088	-	-
60 DA2A	90	0.041	-	-
90 DA2A	120	0.050	-	-
120 DA2A	150	0.010	-	-
180 DA2A	210	0.007	-	-

Average residues of 2,4-D in the 15-30 cm soil layer were 0.026 ppm (1 DA1A) and 0.03 ppm (1DA2A). Residues were detected in this layer only at these sampling intervals. Likewise, at 1 DA1A, 2,4-D was found in the 45-60 cm layer (0.009 ppm) but was not detected in the layer above this. Therefore, this detection is likely to be due to mechanical mixing and sample contamination. Residues of the metabolites 2,3-DCP and 2,4-DCA were detected only in the top 15 cm layer.

The half-lives of 2,4-D residues following the first and second applications have been calculated based on the equation T1/2 = -ln(2)/m. For the first application, m = -0.121, r^2 = 0.90 and the calculated half-life was 5.7 days. Following the second application, m = -0.023, r^2 = 0.88 and the calculated half-life was 30.5 days.

Table A3.7: Residue Formation and Decline in the Texan Trial (ppm).

Time	Cumulative	2,4-D	2,4-DCP	2,4-DCA
0 DA1A	0	1.234	-	-
1 DA1A	1	1.138	-	-
3 DA1A	3	0.907	0.003	-
7 DA1A	7	0.677	0.012	-
-1 DA2A	25	-	-	-
0 DA2A	26	0.438	0.013	-
1 DA2A	27	0.383	0.012	-
3 DA2A	29	0.207	-	-
7 DA2A	33	0.135	-	-
15 DA2A	41	0.093	-	-
30 DA2A	56	0.054	-	-
64 DA2A	90	0.004	-	-
94 DA2A	120	-	-	-
120 DA2A	146	-	-	-

Residues were almost exclusively found in the top 15 cm of soil. The only exceptions were the detection of 2,4-D in one replicate at a concentration of 0.036 ppm in the 15-30 cm layer at 1 DA1A, and 2,4-DCP in the same soil layer in one replicate at a concentration of 0.011 ppm at 3 DA1A.

Half-lives were calculated by DEH for dissipation curves using the slope derived from the regression model in the equation $T1/2 = -\ln(2)/m$. After the first application, the half-life for 2,4-D was calculated to be 7.9 days (m = -0.0878, r^2 = 0.97) while the half-life after the second application was calculated to be 10.2 days (m = - 0.0679, r^2 = 0.99).

Conclusions:

These studies demonstrate that when pasture is treated with two consecutive broadcast applications of the DMA of 2,4-D up to a total rate of almost 5 kg/ha, 2,4-D, 2,4-DCP and 2,4-DCA are relatively immobile in sandy loam soil. Dissipation half-lives were mainly around 1–1.5 weeks with one significantly longer half-life measured of around 4.5 weeks.

Wheat

Two field studies were provided for application of 2,4-D DMA to wheat applied as WEEDAR Brand 64 Broadleaf Herbicide (46.8% DMA; 38.9% acid equivalent – ae) 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 DMA**	**2,4-D DMA**
Report:	Barney, 1995h.	Silvoy, 1994a
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 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 0-15 cm:

Location	Texture	% Sand	% Silt	% Clay	% OM	CEC (meq/100 g)	pH	% MC
Nth Carolina	Sand	92.8	4.0	3.2	1.22	2.05	5.9	3.99
Colorado	Sandy clay loam	51.2	19.6	29.2	1.44	12.74	8.1	26.75

The bulk density of the North Carolina soil was 1.57 g/cm^3 while that for the Colorado soil was 1.45 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 Colorado study, the first application was made when the wheat was past the joint stage with the second 60 days later. This deviation is noted. It is also noted that application in the North Carolina study occurred when the crop was at the stem extension crop stage (around 38 cm tall). There would be greater interception at the first application than expected if the protocol was followed. However, bare ground studies on wheat use patterns have been performed and these results will be considered separately.

Equipment used was standard commercial ground application equipment sprayed in a nominal volume of 10 gallons/acre (around 95 L/ha) except the first application in the Colorado trial where application was using a backpack. The target application rate was 1.4 kg ai/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, 7, 15, 32 and 60 (three days prior to the second application) DAT for the first application, and 0, 1, 4, 7, 15, 36, 58, 92, 120 and 182 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 0, 1, 3, 7, 15, 30, 60, 90, 120, 180, 360 and 540 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, 2,4-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. The first contained 2,4-DCP and 2,4-DCA. The second fraction, containing 2,4-D, was concentrated and methylated to form the methyl ester (2,4-D ME), then partitioned (petroleum ether/hexane). The two fractions were then 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 67% (application 1) and 88% (application 2).

Recoveries achieved on analysis of the application monitors at the Colorado trial were 100% (application 1) and 91% (application 2).

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

Findings:	Table A.3.8 summarises the residue formation and decline of 2,4-D and metabolites in the North Carolina trial which Table A3.9 does the same for the Colorado trial.

Table A3.8: Residue Formation and Decline in the North Carolina Trial (ppm).

Time	Cumulative	2,4-D	2,4-DCP	2,4-DCA
0 DA1A	0	0.195	-	-
1 DA1A	1	0.232	0.015	-
3 DA1A	3	0.251	0.019	-
7 DA1A	7	0.081	0.021	0.003
15 DA1A	15	0.023	0.008	0.003-
32 DA1A	32	-	-	-
-3 DA2A	60	-	-	-
0 DA2A	63	0.262	0.012	-
1 DA2A	64	0.278	0.017	-
4 DA2A	67	0.098	0.014	-
7 DA2A	70	0.172	0.016	-
15 DA2A	78	0.009	-	-
30 DA2A	94	-	-	-
62 DA2A	126	-	-	-
91 DA2A	155	-	-	-

No residues of any analyte were found at any sampling time below the top 15 cm soil layer with the one exception of 0.03 ppm 2,4-D being found in one replicate of the 15-30 cm layer at 7 DA1A (average of 0.010 ppm in this layer for three replicates).

Least squares linear regression was performed on the 2,4-D residue data 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 $T1/2 = -\ln(2)/m$. For the 32 DA1A and 15 DA2A residue levels, half the LOQ was used (0.005 ppm).

2,4-D exhibited a half-life of 5.5 days ($r^2 = 0.96$) after the first application and 2.7 days ($r^2 = 0.87$) after the second application.

Table A3.9: Residue Formation and Decline in the Colorado Trial (ppm).

Time	Cumulative	2,4-D	2,4-DCP	2,4-DCA
0 DA1A	0	0.242	-	-
1 DA1A	1	0.223	-	-
4 DA1A	4	0.365	-	-
7 DA1A	7	0.292	-	-
16 DA1A	16	0.022	-	-
30 DA1A	30	0.011	-	-
-1 DA2A	57	0.007	-	-
0 DA2A	58	0.201	0.012	-
1 DA2A	59	0.306	0.014	-
3 DA2A	61	0.233	-	-
7 DA2A	65	0.318	-	-
14 DA2A	72	0.08	-	-
29 DA2A	87	-	-	-
60 DA2A	118	-	-	-

No residues of any analyte were found at any sampling time below the top 15 cm soil layer with the one exception of 0.01 ppm 2,4-D being found in one replicate of the 15-30 cm layer at 3 DA1A (average of 0.003 ppm in this layer for three replicates).

Least squares linear regression was performed on the 2,4-D residue data 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.

According to the authors, 2,4-D exhibited a half-life of 5.7 days ($r^2 = 0.86$) after the first application. However, 2,4-D was detected in one replicate at 0.01 ppm and a second at 0.011 ppm at 57 DA1A to give a mean residue at this time of 0.007 ppm. This value was ignored in the study calculations and if used, a half-life of 9.4 days is predicted ($r^2 = 0.79$). The authors determined a half-life of 5.1 days ($r^2 = 0.90$) after the second application. However, they used half the LOQ (0.005 ppm) as the residue level for 29 DA2A. No residues were detected at this time, so the reason for this is unclear except it results in a much better correlation coefficient for least squares regression. Based on the residue data provided in the above table, the half-life up until 14 DA2A is calculated to be 9.6 d ($r^2 = 0.54$).

Conclusions:

These studies demonstrate that when wheat is treated with two consecutive broadcast applications of the DMA of 2,4-D up to a total rate of 2.8 kg ae/ha, 2,4-D, is relatively immobile in sand and sandy clay loam soil. Dissipation half-lives were less than 10 days at both field sites.

Turf

Two field studies were provided for application of 2,4-D DMA to turf applied as WEEDAR Brand 64 Broadleaf Herbicide (46.8% DMA; 38.9% acid equivalent – ae) 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 DMA**	**2,4-D DMA**
Report:	Barney, 1995i	Hatfield, 1995j
Guidelines:	US-EPA Subdivision N; 164-1	US-EPA Subdivision N; 164-1
GLP:	Yes	Yes
Test System		
Location:	Rowland, North Carolina.	Tulare County, California.
Plot sampling and irrigation:	5 sampling areas in the treated plot; 3 in the UTC. The treated plots were located downslope from the UTC. Irrigation was applied as needed to the test areas to assure 110-120% or more of each monthly precipitation average found during the previous 10-30 year period.	

Soil texture in the top 0-15 cm:

Location	Texture	% Sand	% Silt	% Clay	% OM	CEC (meq/100 g)	pH	% MC
Nth Carolina	Sand	92.8	4.0	3.2	1.43	2.03	6.8	3.85
California	Sandy loam	68	27	5	1.2	8.6	7.7	11.4

The bulk density of the North Carolina soil was 1.55 g/cm^3 while that for the Californian soil was 1.10 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. Turf height at both applications in the North Carolina trial was about 10 cm.
	Equipment used was standard commercial ground application (tractor mounted) equipment sprayed in a nominal volume of 30 gallons/acre (around 280 L/ha). The boom height was 15-18 inches (38-46 cm) above the ground. The target application rate was 2.24 kg ae/ha in the North Carolina trial and 2.46 kg ae/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 (except in the Californian trial where irrigation water samples were not collected at two of the required intervals).
Sampling	**Soil sampling** was undertaken according to the following regime:
	North Carolina: soil samples were collected from the untreated and treated plots at –10, 0, 1, 2, 5, 14 and 20 (one day prior to the second application) DAT for the first application, and 0, 1, 2, 5, 14, 35, 64, 92 and 121 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 (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 application 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: According to the protocol, **grass** clippings and **thatch** samples (from the 0-15 cm soil layer) were to be obtained following the same sampling regime as that for soil above. The body of the report does not discuss sampling of grass and thatch. However, this did occur as analytical methods and residues determined are provided.

Analysis   Soil residue data were generated for 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 achieved on analysis of the application monitors at the North Carolina trial were 112% (application 1) and 99.5% (application 2). These recoveries were the mean of 15 separate monitors corrected for overall recovery.

Recoveries achieved on analysis of the application monitors at the California trial were 83% (application 1) and 79% (application 2).

These recoveries were the mean of 9 separate monitors corrected for overall recovery.

Findings: **SOIL**. Table A3.10 summarises the residue formation and decline in the North Carolina trial while Table A3.11 does the same for the Californian trial.

Table A3.10: Residue Formation and Decline in Soil in the North Carolina Trial (ppm).

Time	Cumulative	2,4-D	2,4-DCP	2,4-DCA
0 DA1A	0	0.764	-	-
1 DA1A	1	0.747	0.006	-
2 DA1A	2	0.448	0.009	-
5 DA1A	5	0.090	0.008	0.013
14 DA1A	14	0.023	-	-
-1 DA2A	20	0.013	-	-
0 DA2A	21	0.860	-	-
1 DA2A	22	0.556	0.007	-
2 DA2A	23	0.128	0.007	-
5 DA2A	26	0.090	-	-
14 DA2A	35	0.009	-	-
35 DA2A	56	-	-	-
64 DA2A	85	-	-	-

Soil residues of 2,4-D and the 2,4-DCP and 2,4-DCA degradates were found only in the top 15 cm of soil at all sampling points following both applications.

Least squares linear regression was performed with both the 2,4-D acid 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 $T_{1/2} = -\ln(2)/m$.

2,4-D exhibited half-lives of 3.3 days ($r^2 = 0.92$) after the first application and 2.3 days ($r^2 = 0.91$) after the second.

Table A3.11: Residue Formation and Decline in Soil in the Californian Trial (ppm).

Time	Cumulative	2,4-D	2,4-DCP	2,4-DCA
0 DA1A	0	0.084	-	-
1 DA1A	1	0.043	-	-
3 DA1A	3	0.028	-	-
7 DA1A	7	0.368	-	0.009
14 DA1A	14	0.111	0.003	0.027
-1 DA2A	20	0.020	-	0.018
0 DA2A	21	0.123	-	0.014
1 DA2A	22	0.093	-	0.014
3 DA2A	24	0.076	-	0.013
7 DA2A	28	0.209	-	0.021
14 DA2A	35	0.023	-	0.015
30 DA2A	51	-	-	-
60 DA2A	81	-	-	-

Residues of 2,4-D declined progressively for the first three days following application. The half-life over this period was 2.0 days ($r^2 = 0.90$). However, much higher residues were found at day 7 where they again dissipated in a linear fashion with a half-life from this point onwards of less than one day ($r^2 = 0.99$) until the second application. It is difficult to rationalise the increase in the residues found at 7 days (some 13 times higher than 3 DA1A) when no build up was observed prior to this. While the report states that daily climatological data were collected, only monthly rainfall/irrigation values seem to be provided. Therefore, it is impossible to say whether rainfall may have impacted on the data.

In addition, movement through the soil profile was observed. Average residues of 2,4-D in the 15-30 cm layer ranged from 0.004 ppm at 7 DA2A to 0.037 ppm at 14 DA1A. Further, at 7 DA2A, 2,4-D was detected down to 75 cm. Again, at 14 DA2A, 2,4-D was detected in alternating depth increments from 45-120 cm that was theorised as being due to mechanical mixing and sample contamination.

Residues of the metabolite 2,4-DCA were detected only in the 0-15 cm layer from 7 DA1A to 14 DA2A with detectable residues of 2,4-DCP only being found at 14 DA1A in the top 15 cm.

Linear regression analysis was performed to determine half-lives. Due to the sudden increase in residues found at days 7 and 14 following the first application, and a similar sudden increase at day 7 following the second application, the linear regressions showed very poor correlation coefficients. The study reports a half-life of 21.9 days ($r^2 = 0.03$) following the first application and 7.5 days ($r^2 = 0.41$) after the second applicaiton.

Findings: **Grass and Thatch**

Table A3.12: 2,4-D Mean Residues (ppm) in Grass and Thatch.

North Carolina Trial		Californian Trial		
Time	Grass	Time	Grass	Thatch
0 DA1A	15.6	0 DA1A	120.6	4.63
1 DA1A	13.5	1 DA1A	138.0	2.03
2 DA1A	12.1	3 DA1A	85.83	3.16
5 DA1A	5.2	7 DA1A	28.07	1.90
14 DA1A	3.3	14 DA1A	13.60	0.35
-1 DA2A	1.4	-1 DA2A	15.63	0.39
0 DA2A	25.3	0 DA2A	240.3	3.76
1 DA2A	45.4	1 DA2A	149.3	1.97
2 DA2A	31.7	3 DA2A	152.3	3.38
5 DA2A	13.1	7 DA2A	22.4	1.40
14 DA2A	3.8	14 DA2A	20.0	1.24
35 DA2A	1.4	30 DA2A	8.40	0.00
64 DA2A	<1.0	60 DA2A	1.89	0.00

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

Based on mean residues, the half-life of 2,4-D in grass in the North Carolina trial was calculated to be 6.4 days ($r^2 = 0.90$) after the first application and 7.7 days ($r^2 = 0.83$) 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 10 cm high at both applications. 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 ($r^2 = 0.85$) after the first application and 9.3 days ($r^2 = 0.85$) after the second application.

Thatch residues in the Californian study were significantly less than those found on grass. Based on mean residues, the half-life of 2,4-D in thatch was calculated to be 5.5 days ($r^2 = 0.87$) after the first application and 9.8 days ($r^2 = 0.64$) after the second application.

Conclusions:

These studies demonstrate that when turf is treated with two consecutive broadcast applications of the DMA of 2,4-D up to a total rate of almost 5 kg ae/ha (combined rate), 2,4-D is relatively immobile in sand, but appears somewhat more mobile in sandy loam soil. Dissipation in the sand was quick with a half-life of 3.3 days or less following both applications. Some concerns exist with the residues detected in the Californian study (sandy loam). The overall dissipation half-life from the first application was 22 days while it was 7.5 days following the second application.

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. Dissipation was similar to that for grass with half-lives less than 10 days following both applications.

Granules

Two field studies were provided for application of 2,4-D DMA in the granular form, Clean Crop Weed and Feed (1.0% ae granular). Both trials were undertaken in North Dakota, 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 DMA**	**2,4-D DMA**
Report:	Hatfield, 1995k	Hatfield, 1995l
Guidelines:	US-EPA Subdivision N; 164-1	US-EPA Subdivision N; 164-1
GLP:	Yes	Yes
Test System	Bare Ground	Turf
Location:	Grand Forks County, North Dakota	Grand Forks County, North Dakota
Plot sampling and irrigation:	5 sampling areas in the treated plot; 3 in the UTC. The treated plots were located 30-60 m downslope from the UTC. In both trials (trial plots were adjacent to each other), no irrigation was provided during the trial. The cumulative rainfall for the trial period was 158% of the norm.	

Soil texture (top 15 cm)

Situation	Texture	% Sand	% Silt	% Clay	% OM	CEC (meq/100 g)	pH	% MC
Bare Ground	Loam	51	29	20	3.3	31.4	7.7	26.2
Turf	Sandy Loam	61	33	6	6.4	24.6	6.4	28.7

The bulk density of both soils was between 1.06 and 1.011 g/cm^3

Experimental treatments:	For both tests, the treated plots received two applications with a target rate of 2.46 kg/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 13 inches (33 cm) above the row.
	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.

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 116 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.

As explained in the study protocol for 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, 2,4-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. The first contained 2,4-DCP and 2,4-DCA. The second fraction, containing 2,4-D, was concentrated and methylated to form the methyl ester (2,4-D ME), then partitioned (petroleum ether/hexane). The two fractions were then combined into a single solution for chromatographic analysis.

Grass and thatch samples were analysed for 2,4-D 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: For applications 1 and 2, rates of 112% and 119% of the target rate were achieved respectively in both trials. Rationale for these higher rates as described in the protocol deviations was that limited test substance was available resulting in the applicator being calibrated with a different material which may have been affected by high humidity. Additionally, grinding of the test substance during application may have affected the flow rate.

Findings: **SOIL**. Table A3.13 summarises the residue formation and decline in the bare ground trial while Table A3.14 does the same for the turf trial.

Table A3.13: Residue Formation and Decline in Soil in the Granules Bare Ground Trial (ppm).

Time	Cumulative	2,4-D	2,4-DCP	2,4-DCA
0 DA1A	0	1.384	-	-
1 DA1A	1	1.503	-	-
3 DA1A	3	1.34	0.014	-
7 DA1A	7	1.002	0.021	0.022
14 DA1A	14	1.33	0.021	0.023
-1 DA2A	20	0.501	0.025	0.029
0 DA2A	21	1.209	0.020	0.024
1 DA2A	22	1.563	0.025	0.026
3 DA2A	24	1.933	0.023	0.029
7 DA2A	28	1.667	0.021	0.026
14 DA2A	35	0.890	0.020	0.019
30 DA2A	51	0.269	0.003	0.005
60 DA2A	81	0.028	0.004	0.008
90 DA2A	111	0.011	-	-
116 DA2A	137	0.019	-	0.007

Average residues of 2,4-D in the 15-30 cm depth were detected on 3 DA1A at 0.022 ppm, 1 DA2A at 0.02 ppm, 3 DA2A at 0.017 ppm and 7 DA2A at 0.004 ppm. At 7 DA1A, 2,4-D was detected down to 75 cm, however, this is probably due to mechanical mixing and sample contamination as the 15-30 cm layer did not have any residues detected. Following the second application (1 DA2A), residues of 2,4-D were detected throughout the 0-60 cm soil depth between average residues of 0.008-0.02 ppm. At 3 DA2A, detections were also observed down to the 75 cm layer but subsequent sampling intervals had no detectable residues below the 15-30 cm layer.

Detectable residues of 2,4-DCP were found only in the 0-15 cm depth from 3 DA1A to 60 DA2A. Small concentrations (0.003-0.009 ppm) of 2,4-DCA were found below 15 cm on three events, namely, 7 DA1A, 1 DA2A and 3 DA2A.

Least squares linear regression was calculated for dissipation curves using the slope derived from the regression model in the equation $T1/2 = -\ln(2)/m$.

Following the first application, dissipation of 2,4-D was 16.7 days ($r^2 = 0.64$) with residues remaining somewhat constant until 14 DA1A. The authors report a dissipation half-life of 11.7 days following the second application ($r^2 = 0.86$). However, they only calculated this based on residues up to 30 DA2A. Residues were detected up until the end of the study (116 DA2A), and if all mean residue data are used, the half-life is 14.7 days ($r^2 = 0.90$). It is not clear from the report why these extra data were ignored.

2,4-DCP increased in residues over the time between the first and second applications. The dissipation half-life following the second application is calculated by DEH to be 19.5 days ($r^2 = 0.74$). This is in contrast to that calculated by the authors of 10.7 days ($r^2 = 0.83$). Again, the authors only calculated up to 14 DA2A. DEH used the additional mean residue data up to 60 DA2A.

2,4-DCA increased in residues over the time between the first and second applications. The dissipation half-life following the second application is calculated by DEH to be 27.2 days ($r^2 = 0.66$). This is in contrast to that calculated by the authors of 12.6 days ($r^2 = 0.89$). Again, the authors only calculated up to 30 DA2A. DEH used the additional mean residue data for 60 DA2A, and it is noted this analyte was still found at 116 DA2A (although none was detected at 90 DA2A).

Table A3.14: Residue Formation and Decline in Soil in the Granules Turf Trial (ppm).

Time	Cumulative	2,4-D	2,4-DCP	2,4-DCA
0 DA1A	0	0.089	-	-
1 DA1A	1	0.232	-	-
3 DA1A	3	0.819	0.022	0.009
7 DA1A	7	0.307	0.029	0.042
14 DA1A	14	0.172	0.023	0.060
-1 DA2A	20	0.044	0.023	0.056
0 DA2A	21	0.102	0.018	0.057
1 DA2A	22	0.110	0.018	0.046
3 DA2A	24	0.742	0.019	0.046
7 DA2A	28	0.071	0.015	0.019
14 DA2A	35	0.016	0.014	0.018
30 DA2A	51	0.004	0.013	0.012
60 DA2A	81	-	-	-
90 DA2A	111	-	-	-
116 DA2A	137	-	-	-

Generally, residues were retained in the top 15 cm. However, on 3 DA1A, 2,4-D was detected at an average of 0.014 ppm in the 15-30 cm depth while at 3 DA2A, it was found at 0.017 ppm and 0.044 ppm in the 15-30 cm and 30-45 cm depths respectively. 2,4-DCP was not detected at any time below the 15 cm layer while 2,4-DCA was found once at a mean concentration of 0.003 ppm in the 30-45 cm layer at 3 DA2A.

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 $T1/2 = -\ln(2)/m$.

After the first application, concentrations of 2,4-D continued to increase until 3 DA1A due to conversion of the test substance into its metabolites. The half-life based on all mean residue data from the first application was 10.3 days ($r^2 = 0.28$). However, if it is calculated from the residue peak at day 3, the dissipation half-life is 1.8 days ($r^2 = 0.96$). From the second application, the dissipation half-life is calculated to be 5.1 days ($r^2 = 0.75$).

Findings: **Grass and Thatch**

Table A3.15: 2,4-D Mean Residues (ppm) in Grass and Thatch from Turf Trial.

Time	Grass	Thatch
0 DA1A	134.67	21.77
1 DA1A	61.00	15.40
3 DA1A	6.08	8.55
7 DA1A	5.73	5.40
14 DA1A	1.79	4.35
-1 DA2A	-	-
0 DA2A	64.30	14.33
1 DA2A	37.77	15.03
3 DA2A	41.77	8.28
7 DA2A	13.24	5.21
14 DA2A	1.26	0.11
30 DA2A	-	-
60 DA2A	-	-

In grass clippings the dissipation half-life following the first application was calculated to be 2.5 days for 2,4-D ($r^2 = 0.76$) with residues peaking at the day of application. Following the second application, residues again appeared to have peaked on the day of application and the half-life was calculated to be 2.5 days ($r^2 = 0.97$).

In thatch the dissipation half-life following the first application was calculated to be 6.4 days for 2,4-D ($r^2 = 0.81$) with residues peaking at the day of application. Following the second application, residues appeared to peak after the first day and the half-life was calculated to be 2.0 days ($r^2 = 0.92$).

Conclusions:

Conversion of DMA 2,4-D to the 2,4-D acid appeared to occur relatively quickly with peak concentrations in soil being found within a few days of application. Dissipation for half of 2,4-D from soil when applied in granular form to bare ground occurred in about 14.7-16.7 days. The analytes 2,4-DCP and 2,4-DCA dissipated from bare ground with half-lives of around 19 and 27 days respectively.

When applied as granules to turf, the dissipation of 2,4-D from soil was somewhat faster with half-lives of 5.1-10.3 days. 2,4-D residues in grass and thatch generally appeared to peak on the day of application with one exception in thatch where the peak occurred one day after application. Dissipation half-lives following both applications in both grass and thatch were 2.5 days or less except that following the first application where the dissipation half-life in thatch was calculated to be 6.4 days.

Bare Soil

Seven field studies were provided for application of 2,4-D DMA applied as WEEDAR Brand 64 Broadleaf Herbicide (46.8% DMA; 38.9% acid equivalent – ae) 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 DMA	
Report	**Author**	**Situation**
1 of 7	Hatfield, 1995m	Bare ground in California
2 of 7	Barney 1995j	Bare ground in North Carolina – Turf use pattern
3 of 7	Silvoy, 1994b	Bare ground in Colorado – Wheat use pattern
4 of 7	Hatfield, 1995n	Bare ground in North Dakota – Wheat use pattern
5 of 7	Barney, 1995k	Bare ground in North Carolina – Wheat use pattern
6 of 7	Hatfield, 1995o	Bare ground in Nebraska – Corn use pattern
7 of 7	Hatfield, 1995p	Bare ground in Ohio – Corn use pattern

Plot sampling and irrigation: Treated plots consisted of 3 replicates with 5 sampling points in each while there was one replicate with 3 sampling points in the UTC (studies 1, 4, 6 and 7), or 5 sampling sub-plots with three replicate samples from each in the treated plots and three sampling sub-plots with a single core sampled in the UTC (studies 2, 3 and 5).

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 120% of the 10 year average (tests 1, 4, 6 and 7) or

110% of the 10 year average (tests 2, 3, and 5)

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	Sandy loam	70	25	5	1.3	7.9	8.6	11.9	1.15
2 of 7	Sand	97.8	0.0	5.2	0.77	6.9	1.72	3.0	1.62
3 of 7	Sand clay loam	53.6	20.0	26.4	1.77	7.8	11.97	20.77	1.41
4 of 7	Sandy loam	56	30	14	2.9	7.2	25.2	24.1	1.12
5 of 7	Sand	88.8	6.0	5.2	1.05	6.5	2.12	3.78	1.62
6 of 7	Silt loam	20	559	21	3.5	6.7	19.9	27.2	1.14
7 of 7	Silty clay loam	19	51	30	2.3	7.1	16.7	27.8	1.12

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 A3.16: Experimental Treatments for Bare Soil Field Dissipation Studies.

Report	Situation	No. applic.	Rate/app (kg ae/ha)	Days apart	L/ha	Boom height
1 of 7	Bare	2	2.46	21	280	45 cm
2 of 7	Turf	2	2.24	21	280	48 cm
3 of 7	Wheat	2	1.4	60	100	Not stated
4 of 7	Wheat	2	1.54	61	47	50-55 cm
5 of 7	Wheat	2	1.4	63	93.5	48-51 cm
6 of 7	Corn	4	2.46; 1.23; 0.62; 1.85	15; 30; 94	93.5	20
7 of 7	Corn	4	2.46; 1.23; 0.62; 1.85	19; 34; 85	93.5	30-46

Application was undertaken using standard ground based equipment. This generally consisted of tractor mounted booms. However, the first application in study 3 was applied using a backpack as were all four applications in study 7.

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

Table A3.17: Soil Sampling Regime

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, 31, 59, 91 and 120
2 of 7	Turf	1	-10, 0, 1, 2, 5, 14 and 20 (1 day before second application)
		2	0, 1, 2, 5, 14, 35, 64, 92 and 121.
3 of 7	Wheat	1	-1, 0, 1, 3, 7, 16, 30 and 57 (3 days before second application)
		2	0, 1, 3, 7, 14, 29, 60 and 90.
4 of 7	Wheat	1	-1, 0, 1, 3, 7, 14, 30 and 60 (1 day before second appl^n)
		2	0, 1, 3, 7, 14, 30, 60, 90 and 108.
5 of 7	Wheat	1	-1, 0, 1, 3, 7, 15, 32 and 60 (1 day before second appl^n)
		2	0, 1, 4, 7, 15, 36, 58, 92, 120 and 182.
6 of 7	Corn	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, 93 (one day before fourth appl^n)
		4	0, 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, 7, 14, 30, 60, 84 (one day before fourth appl^n)
		4	0, 1, 3, 7, 14, 30, 60, 90, 120, 180 and 210.

Analysis   Soil residue data were generated for 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. The first contained 2,4-DCP and 2,4-DCA. The second fraction, containing 2,4-D, was concentrated and methylated to form the methyl ester (2,4-D ME), then partitioned (petroleum ether/hexane). The two fractions were then 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 A3.18. Following this, Tables A3.19 through to A3.25 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 and certain metabolites through the soil profile.

Application
verification:

Table A3.18: Application Recoveries Based on Monitoring Pad Analysis:

Study	Application	No. Samples	Target rate (kg ae/ha)	Actual rate (kg ae/ha)	% Target
1 of 7	1	9	2.46	1.98	80.5
	2	9	2.46	2.21	89.6
2 of 7	1	15	2.24	2.42	109.5
	2	15	2.24	2.21	98.7
3 of 7	1	15	1.4	1.28	91.4
	2	15	1.4	1.55	110.7
4 of 7	1	9	1.54	1.56	101.3
	2	9	1.54	1.66	107.8
5 of 7	1	15	1.4	1.13	80.7
	2	15	1.4	1.31	93.6
6 of 7	1	9	2.46	1.47	59.7
	2	9	1.23	1.06	86.2
	3	9	0.62	0.60	96.8
	4	9	1.85	1.38	74.6
7 of 7	1	9	2.46	1.84	74.8
	2	9	1.23	1.27	103.2
	3	9	0.62	0.47	75.8
	4	9	1.85	1.65	89.2

Findings: **Residues**

Table A3.19: Residue Formation and Decline in Bare Ground Soil (ppm), Report 1 of 7.

Time	Cumulative	2,4-D	2,4-DCP	2,4-DCA
0 DA1A	0	0.514	-	-
1 DA1A	1	0.255[1,2]	-	-
3 DA1A	3	0.169[3]	-	-
7 DA1A	7	0.412	-	0.008
14 DA1A	14	0.137	-	0.017
-1 DA2A	20	0.036[4]	-	0.023
0 DA2A	21	0.833	-	0.015
1 DA2A	22	1.403[5]	-	0.027
3 DA2A	24	0.817[6]	-	0.028
7 DA2A	28	0.132[7]	-	0.016
14 DA2A	35	0.021[8]	-	0.025
31 DA2A	52	-	-	0.007
59 DA2A	80	-	-	-
91 DA2A	112	-	-	-

1) Found in the 15-30 cm layer at mean residue of 0.004 ppm
2) Found in the 45-60 cm layer at mean residue of 0.011 ppm, not found in the 30-45 cm layer.
3) Found in the 15-30 cm and 30-45 cm layers at mean residues of 0.169 and 0.007 ppm respectively.
4) Found in the 15-30 cm layer at mean residue of 0.018 ppm
5) Found in the 15-30 cm layer at mean residue of 0.028 ppm
6) Found in the 15-30 cm layer at mean residue of 0.041 ppm
7) Found in the 15-30 cm layer at mean residue of 0.02 ppm
8) Found in the 15-30 cm layer at mean residue of 0.01 ppm

Table A3.20: Residue Formation and Decline in Bare Soil (ppm) – Turf Use Pattern, Report 2 of 7.

Time	Cumulative	2,4-D	2,4-DCP	2,4-DCA
0 DA1A	0	0.870	0.004	-
1 DA1A	1	0.842	0.023	-
2 DA1A	2	1.19	0.046	0.011
5 DA1A	5	0.434	0.032	0.021
14 DA1A	14	0.036	0.013	0.006
-1 DA2A	20	0.025	-	-
0 DA2A	21	0.893	0.004	-
1 DA2A	22	0.378[1]	0.018	0.003
2 DA2A	23	0.157	0.015	-
5 DA2A	26	0.033[2]	0.009	-
14 DA2A	35	0.013	-	-
35 DA2A	56	-	-	-
64 DA2A	85	-	-	-
92 DA2A	113	-	-	-

1) Found in one replicate in the 15-30 cm layer at 0.066 ppm (average of 3 replicates = 0.022 ppm)
2) Found in one replicate in the 15-30 cm layer at 0.014 ppm (average of 3 replicates = 0.005 ppm)

Table A3.21: Residue Formation and Decline, Bare Soil Wheat Use Pattern (ppm) – Report 3 of 7.

Time	Cumulative	2,4-D	2,4-DCP	2,4-DCA
0 DA1A	0	0.335	-	-
1 DA1A	1	0.468	-	-
3 DA1A	3	0.317	-	-
7 DA1A	6	0.304	-	-
16 DA1A	16	0.048	-	-
30 DA1A	30	0.012	-	-
-1 DA2A	57	-	-	-
0 DA2A	60	0.353	0.015	-
1 DA2A	61	0.380	0.017	-
3 DA2A	63	0.357	-	-
7 DA2A	67	0.492	-	-
14 DA2A	74	0.047	-	-
29 DA2A	89	-	-	-
60 DA2A	120	-	-	-

No residues of any analyte were found at any sampling time below the top 15 cm depth.

Table A3.22: Residue Formation and Decline, Bare Soil (ppm) – Wheat Use Pattern, 4 of 7.

Time	Cumulative	2,4-D	2,4-DCP	2,4-DCA
0 DA1A	0	0.516	0.007	-
1 DA1A	1	0.415	-	-
3 DA1A	3	0.326	-	-
7 DA1A	7	0.355	0.015	-
14 DA1A	14	0.037^{1}	0.012	0.024
30 DA1A	30	0.0013	-	0.004
-1 DA2A	54	0.005	-	-
0 DA2A	55	0.637	-	-
1 DA2A	56	0.531	-	-
3 DA2A	58	0.211	0.004	-
7 DA2A	62	0.111	-	-
14 DA2A	69	0.070	0.011	-
30 DA2A	85	0.005	-	-
60 DA2A	115	-	-	-

1) Found at mean residue levels of 0.01 ppm in the 15-30, 30-45 and 45-60 cm depths.

Table A3.23: Residue Formation and Decline, Bare Soil (ppm) – Wheat Use Pattern, 5 of 7.

Time	Cumulative	2,4-D	2,4-DCP	2,4-DCA
0 DA1A	0	0.130	0.003	-
1 DA1A	1	0.215	0.017	-
3 DA1A	3	0.189	0.014	-
7 DA1A	7	0.094^{1}	0.014	0.004
15 DA1A	15	-	-	-
32 DA1A	32	-	-	-
-1 DA2A	60	-	-	-
0 DA2A	61	0.299	0.008	-
1 DA2A	62	0.277	0.018	-
4 DA2A	65	0.111^{2}	-	-
7 DA2A	68	0.135	0.016	-
15 DA2A	76	0.009	0.009	-
36 DA2A	97	-	0.012	-
58 DA2A	119	-	-	-

1) Found at mean residue levels of 0.017 ppm in the 15-30 cm depth.
2) Found at mean residue levels of 0.019 ppm in the 15-30 cm depth.

Table A3.24: Residue Formation and Decline, Bare Soil (ppm) – Corn Use Pattern, 6 of 7.

Time	Cumulative	2,4-D	2,4-DCP	2,4-DCA
0 DA1A	0	0.415	-	-
1 DA1A	1	0.666[1]	0.005	-
3 DA1A	3	0.828[2]	0.033	-
7 DA1A	7	0.444[3]	0.27	0.017
-1 DA2A	14	0.183[4]	0.020	0.035
0 DA2A	15	0.508	0.018	0.029
1 DA2A	16	0.621[5]	0.024	0.026
3 DA2A	18	0.685[6]	0.025	0.026
7 DA2A	22	0.515	0.025	0.019
14 DA2A	29	0.045	0.009	0.021
-1 DA3A	44	-	-	0.004
0 DA3A	45	0.239	-	-
1 DA3A	46	0.223	0.004	-
3 DA3A	48	0.175	-	0.004
7 DA3A	52	0.004	-	-
14 DA3A[7]	59	-	-	-
30 DA3A	75	-	-	-
-1 DA4A	138	-	-	-
0 DA4A	139	0.585	-	-
7 DA4A[8]	146	0.322[7]	0.015	-
14 DA4A	153	0.018	-	0.014
30 DA4A	169	-	-	0.004

1)   Found at mean residue levels of 0.009 ppm in the 15-30 cm layer.
2)   Found throughout the soil profile. Mean residue levels 0.017 ppm were found in both the 15-30 cm depth and 105-120 cm depth.
3)   Found throughout the soil profile down to 105 cm (mean residue of 0.018 ppm in the 90-105 cm depth).
4)   Found at mean residue levels of 0.008 and 0.005 ppm in the 15-30 cm and 30-45 cm layer respectively.
5)   Found at mean residue levels of 0.006 ppm in the 15-30 cm layer. Also found at 75-120 cm, but not at layers between 30-75 cm indicating this may be due to contamination.
6)   Found at mean residue levels of 0.012, 0.007 and 0.002 ppm in the 15-30 cm, 30-45 cm and 45-60 cm layer respectively.
7)   Found at mean residue levels of 0.063 ppm in the 15-30 cm layer.

Table A3.25: Residue Formation and Decline, Bare Soil (ppm) – Corn Use Pattern, 7 of 7.

Time	Cumulative	2,4-D	2,4-DCP	2,4-DCA
0 DA1A	0	0.450	-	-
1 DA1A	1	0.904[1]	0.01	-
3 DA1A	3	0.404[2]	0.011	-
7 DA1A	7	0.742	0.021	-
-1 DA2A	17	0.350[3]	0.014	-
0 DA2A	18	0.846	0.019	-
1 DA2A	19	0.895[4]	0.017	-
3 DA2A	21	0.968	0.026	-
7 DA2A	25	0.771[5]	0.02	-
14 DA2A	32	1.098	0.027	-
-1 DA3A	52	0.015	-	0.010[6]
0 DA3A	53	0.196	-	-
1 DA3A	54	0.188	0.012	0.004
7 DA3A	60	-	-	-
14 DA3A	67	-	-	-
-1 DA4A	137	-	-	-
0 DA4A	138	0.467	-	-
1 DA4A	139	0.746	-	-
3 DA4A	141	0.730	0.007	-
7 DA4A	145	0.496	0.008	-
14 DA4A	152	0.442	-	-
30 DA4A	168	0.073	-	-
60 DA4A	197	0.014	-	-
90 DA4A	225	0.006	-	-
120 DA4A	259	0.009	-	-
180 DA4A	319	-	-	-
210 DA4A	349	0.001	-	-

1) Found at mean residue of 0.075 ppm in the 15-30 cm layer.
2) Found at mean residue of 0.015 ppm in the 15-30 cm layer.
3) Found at mean residue of 0.011 ppm in the 15-30 cm layer.
4) Found at mean residue of 0.004 ppm in the 15-30 cm layer.
5) Found at mean residue of 0.005 ppm in the 15-30 cm layer.
6) Found at mean residue of 0.002 ppm in the 15-30 cm layer.

Findings: **Half-lives**

Least squares linear regression was performed with 2,4-D, calculated for dissipation curves using the slope derived from the regression model in the equation $T_{1/2} = -\ln(2)/m$ (in one case, LOG(concentration) was used).

Table A3.26: Half-life 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
2,4-D	App 1	6.8	0.71	3.4	0.95	5.6	0.97	6.5	0.73	2.9	0.87	8.6	0.63	26.1	0.2
	App 2	7.6	0.71	5.8	0.81	5.1	0.66	4.5	0.97	3.0	0.93	3.9	0.78	5.9	0.81
	App 3											1.1	0.88	1.3	0.98
	App 4											4.1	0.89	16	0.88

All calculations have been performed by DEH. Generally, they were in agreement with those provided by the relevant study authors.

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 with 2,4-D.

Conversion of DMA to 2,4-D acid appeared to occur relatively quickly in most cases with residues peaking either on the day of application or within 1 to 3 days of application. Following the second application in study 7 one study, this peak did not occur until 14 days after the application.

The half-life of 2,4-D in the seven bare ground trials varied with a range of 1.1 days to 26.1 days. All except two of the dissipation half-lives were between 1 and 9 days. Of the 18 half-lives determined, the mean half-life was 6.6 days and the 90th percentile half-life was determined to be 10.8 days.

Aquatic Dissipation

Two field studies were provided for application of 2,4-D DMA to ponds. The studies aimed to determine the soil/sediment and water residue dissipation and mobility of 2,4-D and its metabolites in an aquatic environment.

Test Material:	2,4-D DMA	2,4-D DMA
Report:	Hatfield, 1995q	Hatfield, 1995r
Guidelines:	US-EPA Subdivision N; 164-2	US-EPA Subdivision N; 164-2
GLP:	Yes	Yes
Test System		
Location:	Wayne County, North Carolina.	Steele County, North Dakota.
Test product	Weedar 64	Gordon's Amine 400 2,4-D Weed Killer
Formulation	47% ai/39% ae	46.4% ai/38.6% ae
Pond characteristics	One 2.4 acre pond used for the study with maximum length and width of around 205 m and 76 m respectively.	One 14 acre pond used for the study with maximum length and width of around 411 m and 145 m respectively.
	In both tests, according to the protocol, pond depths were measured and water volumes calculated. However, these measurements were not described in the test reports.	
Plot sampling:	Three equally sized subplots were established by linear transects across the width of the pond. At each sampling event, 5 individual sediment cores were collected from each plot. Additionally, pond water samples were collected from the top 90 cm of water.	

Sediment texture – top 5 cm

Location	Texture	% Sand	% Silt	% Clay	% OM	CEC (meq/100 g)	pH	% MC
North Carolina	Sandy loam	55	30	15	3.0	13.9	5.7	22.2
North Dakota	Sandy loam	45	50	5	12.1	30.7	7.7	61.0

The bulk density of the North Carolina soil was 1.03 g/cm^3 while that for the North Dakota soil was 0.59 g/cm^3

Experimental treatments: Two applications were made to the pond sub-surface. The target rate was 46.85 kg ae/ha at both applications with 30-31 days between applications. Application equipment consisted of a boat mounted subsurface applicator with a nominal delivery volume of around 100 L/ha. Tank volumes remaining after each application were measured and the volumes applied to the plot were calculated to verify delivery rates.

At each application, the test substance was sampled for later analysis for verification if necessary. Prior to the first application, bulk samples of sediment and water were collected for spiking procedures. To determine the stability of the analytes throughout the chain of custody, at each application, 12 sediment and water samples field spikes were prepared.

Sampling Soil/sediment and pond water samples were collected from the treated plots according to the following regime:

North Carolina: soil samples were collected from the treated plots at -1, 0, 1, 3, 7, 14, 21 and 29 (one day prior to the second application) DAT for the first application, and 0, 1, 3, 7, 14, 21, 30, 60, 90 and 180 DAT for the second application.

North Dakota: soil samples were collected from the untreated and treated plots at −1, 0, 1, 3, 7, 14, 21 and 30 (one day prior to the second application) DAT for the first application, and 0, 1, 3, 7, 14, 21, 30, 60, 90, 120 and 180 DAT for the second application.

Sediment/soil cores were taken to a depth of 8-12 inches (20-30 cm) according to the protocol. Only the top 20 cm was analysed with 0-5 cm being sediment and 5-20 cm being soil. Cores were segmented into 5 cm layers.

Analysis

The soil samples were analysed for 2,4-D (as acid equivalent), 2,4-DCP, 2,4-DCA, 4-CP and 4-CPA. Samples were vortexed and sonicated in 3 solvent systems. The extracts were decanted, filtered, combined and brought to a known volume. An aliquot of extract (FRACTION A) was combined with hexane, Na_2SO_3, NaCl, 0.5 $\underline{N}$ NaOH (in that order) and 2,4-DCA partitioned to hexane (FRACTION B). Fraction B was concentrated.

FRACTION A was acidified, and 2,4-D, 4-DPA, 2,4-DCP and 4-CP partitioned to Dichloromethane (DCM - FRACTION C). The 2,4-D and 4-CPA were back-partitioned from FRACTION C to 0.25 $\underline{N}$ NaOH (FRACTION E).

FRACTION D was acidified, saturated with salt, and 2,4-D and 4-CPA partitioned twice to ether. Following evaporation of the ether, these analysed were methylated, swamped with water and FRACTION B was added, partitioning the 2,4-D ME and 4-CPA ME in the hexane. An aliquot of the hexane was partitioned from the original FRACTION D and combined with the initial partition. The aqueous layer was discarded. The combined hexane layers were concentrated.

FRACTION E was acidified, saturated with salt and 2,4-DCP and 4-CP partitioned twice to ether. This was concentrated, combined with FRACTION D and concentrated. An aliquot was analysed on a gas chromatograph for quantitation by mass selective detection. The LOQ for all analytes was 0.01 ppm.

Water samples were analysed for 2,4-D (as acid equivalent), 2,4-DCP and 2,4-DCA. Residues were extracted by sonicating in three solvent systems. The combined extracts were diluted with water, acidified and concentrated on a solid phase extraction cartridge. The cartridge was eluted with two solvent systems. The first eluate contained 2,4-DCP, 2,4-DCA and 4-CP. The second, containing 2,4-D and 4-CPA was methylated, partitioned into petroleum ether and combined with the first fraction. The combined solution was concentrated into hexane and analysed by GC using a mass selective detector. The LOQ was 0.001 ppm.

Results and Discussion

Application verification:

The rate applied for applications 1 and 2 in North Carolina were 97 and 108% respectively while 100% application was achieved at both applications in the North Dakota trial. Application rates were verified as explained above using the measureback method. The higher rate for application 2 in the North Carolina study is noted in the protocol deviations as being due to malfunction of the application equipment.

Findings:

Tables A3.27 and A3.28 describes the pattern of residue formation and decline in the North Carolina trial in sediment and water respectively, while Tables A3.29 and A3.30 do the same for the North Dakota trial in sediment and water respectively.

Table A3.27: Residue Formation and Decline in Sediment in the North Carolina Trial (ppm).

Time	Cumulative	2,4-D	2,4-DCP	4-CP
0 DA1A	0	1.14[1]	0.013	-
1 DA1A	1	1.24[2]	0.038	-
3 DA1A	3	1.26[3]	0.098	-
7 DA1A	7	1.45[4]	0.417	-
14 DA1A	14	0.79[5]	0.477[6]	<0.017
21 DA1A	21	0.225[7]	0.155[8]	0.019
-1 DA2A	29	0.103[9]	0.040	0.010
0 DA2A	30	0.622[10]	0.044	0.020
1 DA2A	31	1.77[11]	0.339[12]	0.306[13]
3 DA2A	33	0.796[14]	0.139[15]	0.545[16]
7 DA2A	37	0.137[17]	0.069	0.259[18]
14 DA2A	44	0.013	-[18]	-
21 DA2A	51	-	-	-

1) Found at mean residues of 0.95 ppm and 0.014 ppm in the 5-10 and 10-15 cm depths respectively.
2) Found throughout the soil profile with mean residues of 0.016 ppm in the 15-20 cm layer.
3) Found at mean residues of 0.134 ppm and 0.016 ppm in the 5-10 and 10-15 cm depths respectively.
4) Found throughout the soil profile with mean residues of 0.006 ppm in the 15-20 cm layer.
5) Found throughout the soil profile with mean residues of 0.005 ppm in the 15-20 cm layer.
6) Found throughout the soil profile with mean residues of 0.005 ppm in the 15-20 cm layer.
7) Found throughout the soil profile with mean residues of 0.021 ppm in the 15-20 cm layer.
8) Found throughout the soil profile with mean residues of 0.017 ppm in the 15-20 cm layer.
9) Found at mean residues of 0.021 ppm and <0.007 ppm in the 5-10 and 10-15 cm depths respectively.
10) Found throughout the soil profile with mean residues of 0.0124 ppm in the 15-20 cm layer.
11) Found at mean residues of 0.729 ppm and 0.083 ppm in the 5-10 and 10-15 cm depths respectively.
12) Found at mean residues of 0.167 ppm and 0.038 ppm in the 5-10 and 10-15 cm depths respectively.
13) Found at mean residues of 0.047 ppm and 0.008 ppm in the 5-10 and 10-15 cm depths respectively.
14) Found at mean residues of 0.039 ppm and 0.006 ppm in the 5-10 and 15-20 cm depths respectively.
15) Found at mean residues of 0.011 ppm and 0.007 ppm in the 5-10 and 10-15 cm depths respectively.
16) Found at mean residues of 0.017 ppm in the 5-10 cm layer.
17) Found at mean residues of 0.019 ppm in the 5-10 cm layer.
18) Found at mean residues of 0.007 ppm in the 5-10 cm layer.
19) Found at mean residues of 0.029 ppm in the 5-10 cm layer.

No residues of 2,4-DCA or 4-CPA were found at any sampling event. From 21 DA2A, no residues of any analyte were found with the exception of 0.011 ppm 2,4-DCP found in the 0-5 cm depth at 90 DA2A.

There was obvious movement of 2,4-D through the soil profile, and to a lesser extent, movement of 2,4-DCP and 4-CP. As 20 cm was the maximum depth tested, it is impossible to determine if movement went significantly below this.

The half-lives of 2,4-D residues following the first and second applications have been calculated based on the equation $T_{1/2} = -\ln(2)/m$. For the first application, $m = -0.0877$, $r^2 = 0.89$ and the calculated half-life was 7.9 days. Following the second application, $m = -0.35$, $r^2 = 0.92$ and the calculated half-life was 2.1 days.

Table A3.28: Residue Formation and Decline in Water in the North Carolina Trial (ppm).

Time	Cumulative	2,4-D	2,4-DCP	4-CP	4-CPA
0 DA1A	0	0.652	0.003	-	0.003
1 DA1A	1	1.62	0.004	-	0.005
3 DA1A	3	2.21	0.004	-	0.009
7 DA1A	7	1.77	0.006	-	0.006
14 DA1A	14	1.11	0.006	-	0.005
21 DA1A	21	1.08	0.004	-	0.005
-1 DA2A	29	0.861	0.005	-	0.003
0 DA2A	30	2.80	0.006	-	0.012
1 DA2A	31	2.60	0.004	-	0.012
3 DA2A	33	1.75	0.003	-	0.01
7 DA2A	37	0.630	0.002	-	0.005
14 DA2A	44	0.166	0.003	0.003	0.003
21 DA2A	51	0.011	-	0.001	-
30 DA2A	60	-	-	-	-

No residues of 2,4-DCA were detected at any sampling point.

The authors report a half-life after the first application of 19.7 days ($r^2 = 0.91$). This appears to be taken from when 2,4-D residues peaked at 3 DA1A. If all data are used, the half-life is calculated to be 51 days. However, the correlation is very poor ($r^2 = 0.15$). Dissipation from the second application supports the faster half-life as no residues were detected after 21 DA2A. The half-life from the second application is calculated to be 2.7 days ($r^2 = 0.98$).

Table A3.29: Residue Formation and Decline in Sediment in the North Dakota Trial (ppm).

Time	Cumulative	2,4-D	2,4-DCP
0 DA1A	0	0.253[1]	0.043
1 DA1A	1	0.610[2]	0.022
3 DA1A	3	0.350[3]	
7 DA1A	7	0.365[4]	0.030
14 DA1A	14	1.22[5]	0.035
21 DA1A	21	0.922[6]	0.141
-1 DA2A	30	0.781[7]	0.139[8]
0 DA2A	31	1.04[9]	0.185[10]
1 DA2A	32	1.45[11]	0.097
3 DA2A	34	1.63[12]	0.132
7 DA2A	38	1.59[13]	0.147
14 DA2A	45	1.59[14]	0.174
21 DA2A	52	1.59[15]	0.189
30 DA2A	61	1.59[16]	0.400
60 DA2A	91	0.068[17]	0.144[18]
90 DA2A	121	0.083	0.222[19]
120 DA2A	151	0.057[20]	0.256[21]
180 DA2A	211	0.016[22]	0.141[23]

1) Found at mean residues of 0.010 ppm in the 15-20 cm layer.
2) Found at mean residues of 0.011 ppm and 0.006 ppm in the 5-10 and 15-20 cm depths respectively.
3) Found at mean residues of 0.006 ppm and 0.016 ppm in the 5-10 and 15-20 cm depths respectively.
4) Found at mean residues of 0.025 ppm in the 5-10 cm layer.
5) Found at mean residues of 0.057 ppm in the 5-10 cm layer.
6) Found throughout the soil profile with mean residues of 0.005 ppm in the 15-20 cm layer.
7) Found throughout the soil profile with mean residues of 0.022 ppm in the 15-20 cm layer.
8) Found at mean residues of 0.008 ppm in the 5-10 cm layer.
9) Found throughout the soil profile with mean residues of 0.020 ppm in the 15-20 cm layer.
10) Found at mean residues of 0.011 ppm in the 5-10 cm layer.
11) Found at mean residues of 0.098 ppm and 0.048 ppm in the 5-10 and 15-20 cm depths respectively.
12) Found throughout the soil profile with mean residues of 0.041 ppm in the 15-20 cm layer.
13) Found at mean residues of 0.067 ppm and 0.007 ppm in the 5-10 and 15-20 cm depths respectively.
14) Found throughout the soil profile with mean residues of 0.022 ppm in the 15-20 cm layer.
15) Found at mean residues of 0.103 ppm and 0.015 ppm in the 5-10 and 15-20 cm depths respectively.
16) Found at mean residues of 0.068 ppm in the 5-10 cm layer.
17) Found at mean residues of 0.026 ppm in the 5-10 cm layer.
18) Found at mean residues of 0.018 ppm in the 5-10 cm layer.
19) Found at mean residues of 0.222 ppm in the 5-10 cm layer.
20) Found at mean residues of 0.015 ppm in the 5-10 cm layer.
21) Found at mean residues of 0.021 ppm in the 5-10 cm layer.
22) Found at mean residues of 0.040 ppm in the 5-10 cm layer.
23) Found at mean residues of 0.105 ppm in the 5-10 cm layer.

Residues of 4-CP were only encountered once at mean levels of 0.007 ppm in the 10-15 cm soil depth at 60 DA2A. Residues of 4-CP were only encountered once at mean levels of 0.007 ppm in the 5-10 cm soil layer at 21 DA2A. No residues of 2,4-DCA were found throughout the study.

There was obvious movement of 2,4-D through the soil profile, and to a lesser extent, movement of 2,4-DCP. As 20 cm was the maximum depth tested, it is impossible to determine if movement went significantly below this. From 30 DA2A, no movement for 2,4-D or 2,4-DCP was detected below the top 10 cm of sediment/soil.

Residues of 2,4-D in sediment remained elevated after the first application up until the second. A negative correlation was observed, that is, the half-life was calculated to be –19.8 days. Following the second application, the dissipation of 2,4-D followed first-order kinetics. Residues were still found up to 180 days after the second application and the dissipation half-life was calculated to be 29.5 days ($r^2 = 0.80$).

Table A3.30: Residue Formation and Decline in Water in the North Dakota Trial (ppm).

Time	Cumulative	2,4-D	2,4-DCP	4-CPA
0 DA1A	0	4.65	0.004	0.008
1 DA1A	1	4.78	0.005	0.009
3 DA1A	3	2.66	0.007	0.005
7 DA1A	7	2.47	0.007	-
14 DA1A	14	1.61	0.009	-
21 DA1A	21	1.09	0.008	-
-1 DA2A	30	1.15	0.010	-
0 DA2A	31	3.14	0.004	0.008
1 DA2A	32	3.40	0.005	0.007
3 DA2A	34	3.30	0.010	0.006
7 DA2A	38	2.68	0.008	0.006
14 DA2A	45	2.59	0.009	0.006
21 DA2A	52	2.08	0.010	0.006
30 DA2A	61	1.52	0.007	0.005
60 DA2A	91	0.003	-	0.005

No residues of 2,4-DCA or 4-CP were detected at any sampling point.

The authors report a half-life after the first application of 13.9 days ($r^2 = 0.85$). The half-life from the second application is calculated to be 6.5 days ($r^2 = 0.82$).

Conclusions:

In North Carolina, 2,4-D did not persist in the sediments with a dissipation half-life of 7.9 days after the first application and 2.1 days after the second. This chemical was somewhat more persistent in the North Dakota pond with a sediment dissipation half-life not able to be calculated following the first application and a dissipation half-life following the second application approaching 20 days. The longer persistence is not surprising in this colder climate. Significant movement through the sediment and underlying soil was observed in both studies down to 20 cm (maximum depth tested). However, from 7-30 days after the second application, no movement below the 5-10 cm depth was observed.

Dissipation from water was slower in both trials after the first application (14-20 day half-lives) while it was faster following the second application ($t\frac{1}{2} = 3$-6.5 days).

A further aquatic dissipation study was undertaken to observe behaviour of 2,4-D following application of 2,4-D DMA to a rice paddy.

Test Material:	**2,4-D DMA**
Report:	Barney, 1994
Guidelines:	US-EPA Subdivision N; 164-2
GLP:	Yes

Test System

Location:	Ville Platte, Louisiana.
Test product	DMA 4 (Formulation)
Formulation	46.3% ai/38.4% ae

Plot sampling: The trial consisted of an untreated control plot (UTC) and a treated plot containing 3 and 5 sampling areas respectively.

Sediment texture – top 10 cm

Location	Texture	% Sand	% Silt	% Clay	% OM	CEC (meq/100 g)	pH	% MC
Louisiana	Silt loam	11.2	70.0	18.8	1.21	9.00	6.3	30.45

The bulk density was 1.40 g/cm^3

Experimental treatments: The spray mixture was applied by air using a fixed wing aircraft with equipment calibrated immediately before application. Spray nozzles of the size recommended for an aerial 10 gallon/acre (93.5 L/ha) volume were installed in the boom. The single application was made at a targeted rate of 1.68 kg ae/ha at the green ring stage of growth with a canopy height of 63.5-76 cm. At application, the boom height was 10-15 feet (3-4.6 m) above the canopy.

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 flooding event, for analysis.

Sampling   Soil samples were collected at –4, 0, 1, 3, 7, 15, 30, 45, 60, 90, 120 and 180 DAT. Cores were taken to a depth of 12 inches (30 cm) except for the 0 day sampling interval when samples were taken to a depth of 10 cm.

Water samples were collected at –4, 0, 1, 3, 5, 7, 15 and 30 DAT. These were taken before soil cores to prevent unnecessary contamination from disturbed sediment. At each sampling time, 3 sub samples were collected from each of the 3 different locations along the same sampling lines. The 3 sub samples were composited into one sample. There were a total of 5 treated composited samples and 1 untreated control sample per sampling interval.

Analysis   Soil residue data were generated for 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. The first contained 2,4-DCP and 2,4-
                    DCA. The second fraction, containing 2,4-D, was concentrated and
                    methylated to form the methyl ester (2,4-D ME). The two fractions
                    were then combined into a single solution for chromatographic
                    analysis.

                    Water samples were analysed for 2,4-D (as acid equivalent), 2,4-DCP,
                    2,4-DCA, 4-CPA and 4-CP. Residues were concentrated on a solid
                    phase extraction cartridge. The analytes were eluted sequentially from
                    the cartridge using two specific solvent systems to separate the
                    analytes into two fractions. The first contained 2,4-DCP, 2,4-DCA
                    and 4-CP which were chromatographed without derivatisation. The
                    second fraction contained 2,4-D and 4-CPA which were methylated.
                    The two solutions were combined into a single solution for
                    chromatographic analysis.

Results and Discussion

Application   The rate applied was determined through analysis of 15 application
verification: monitoring pads to be 88.7% of the target application rate.

Findings:

Table A3.31: Residue Formation and Decline in Sediment and Water in a Rice Paddy (ppm).

Time	2,4-D - Sediment	2,4-D - Water
0	0.011	1.3717
1	0.014[1]	0.5377
3	-	0.1945
7	-	-
15	-	-
30	-	-

1)   Found at mean residues of 0.013 ppm and 0.012 ppm in the 10-20 and 20-30 cm depths respectively.

In soil, no residues of 2,4-DCP or 2,4-DCA were found at any sampling event. 2,4-D
was only found at 0 and 1 days after treatment. At 1 DAT, it was found throughout the
soil profile down to the maximum 30 cm depth tested.

In water, no residues of 2,4-DCP, 2,4-DCA, 4-CPA or 4-CP were found at any
sampling event.

While dissipation appears to be fast, this may be the result of low application rates
leading to levels below the detection limit. Dissipation half-lives were calculated
based on the equation $T_{1/2} = -\ln(2)/m$. As no residues were found in soil at 3 days after
treatment, it was assumed ½ LOQ residues were available in order to predict a
dissipation curve. This resulted in a dissipation half-life in soil of 2.3 days ($r^2 = 0.71$).

In water, residues were found at days 0, 1 and 3 with no detections after this point.
Based on the 0-3 DAT levels, the dissipation half-life in water was calculated to be 1.1
days ($r^2 = 0.97$).

Conclusions:

Following a single application of 2,4-D DMA to a rice paddy, movement from water to sediment was limited and dissipation half-lives in both sediment and water were observed with values of 2.3 and 1.1 days respectively. These values should be treated with caution as application rates may not have been high enough to adequately detect residues and establish the true dissipation pattern.

Avian Toxicity

Acute

Only one standard study was submitted to the APVMA for review. In addition, several non-standard tests using non-standard species were also submitted. The results obtained are as follows:

Table A3.32. Summary of Acute Bird Toxicity Results for 2,4-D Salts

Test substance	Species	LD50 (mg ae/kg bw)	Reference
2,4-D DMA	Bobwhite quail	415	Hoxter *et al* 1990
2,4-D DMA[1]	Domestic chicken	1671	Chittibabu, 2002e
2,4-D DMA[1]	Pigeon	334	Chittibabu, 2002f
2,4-D Sodium salt[1]	Domestic chicken	1834	Chittibabu, 2000g
2,4-D Sodium salt[1]	Pigeon	1307	Chittibabu, 2002h

1) Non-Standard test.

Test Substance:	**2,4-D DMA**
Report:	Hoxter *et al*, 1990
Guidelines:	FIFRA Guideline 71-1
GLP:	yes

Test System

The study aimed to evaluate the acute toxicity of 2,4-D DMA when administered to bobwhite quail (*Colinus virginianus*) as a single oral dose. Groups of 10 birds, 21 weeks of age at test initiation, were assigned to each treatment and control group by a random draw. 5 males and 5 females were included in each group. Nominal test concentrations were 0 (control), 125, 250, 500, 1000 and 2000 mg/kg bw. The test substance was dispersed in corn oil.

The primary phases of the study were an 8 week acclimation period, 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 19°C and the average relative humidity was around 26%. The photoperiod was 8 hours of light per day during acclimation and through the study.

All birds were observed at least twice 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. Feed consumption data were reported as an estimate due to unavoidable wastage by the birds. The LD50 value was statistically determined using binomial probability.

Findings

No mortalities were found in the control group or the 125 and 250 mg/kg bw treatment groups. In the 500 mg/kg bw group, one death was observed on the day of treatment (5 hours after treatment) with 50% mortality by day 1. No further mortality was observed in this group. In the 1000 and 2000 mg/kg bw groups, 100% mortality was observed with 50 and 100% occurring by days 0 and 1 respectively in the 1000 mg/kg bw group, and total morality on the day of treatment in the highest treatment group.

There were no overt signs of toxicity in the 125 and 250 mg/kg bw groups. However, in the 500 mg/kg bw group, signs of toxicity were noted on the first morning (around 30 minutes after dosing), and included depression, reduced reaction to external stimuli, wing droop, loss of coordination, prostrate posture, loss of righting reflex, ruffled appearance, lower limb weakness and rapid and shallow respiration.

Similar sub-lethal affects were found in the 1000 and 2000 mg/kg bw treatment groups 15-20 minutes after dosing. In the 1000 mg/kg bw group, the first mortality was found around 1 hour after dosing. In the 2000 mg/kg bw group, the first three mortalities occurred around 30 minutes after dosing with all remaining birds found dead between 45 minutes and 2 hours after dosing.

Compared to the controls, a slight reduction in body weight for birds at the 125 mg/kg bw was observed, and a marked reduction for birds in the next two treatment levels for days 0-3. A corresponding reduction in feed consumption was noted for the females in the 125 mg/kg bw level and for the males and females in the 250 and 500 mg/kg bw levels for this same period.

Conclusion

The acute oral LD50 value for Northern bobwhite exposed to 2,4-D DMA was determined to be 500 mg ac/kg bodyweight (95% CI of 250-1000 mg/kg bw), equating to around 415 mg ae/kg bw. The NOEC was not determined by the study authors. However, based on body weight for days 0-3 after dosing, a NOEC of 125 mg ac/kg bw is considered appropriate.

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

Test System

The acute oral toxicity of 2,4-D DMA salt (59.1% 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 1250, 2500 and 5000 mg/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, 1250, 2500 and 5000 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 2500 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 pale coloured combs after 72 h while birds treated with 2500 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 2,4-D technical to the chicken was calculated to be 2827.2 mg/kg bw with the confidence limits (assumed to be 95%) ranging from 1888-3766 mg/kg bw. In terms of acid equivalence, given the level of 2,4-D in the test formulation, the LD50 was 1671 mg ae/kg bw.

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

Test System

The acute oral toxicity of 2,4-D DMA salt (59.1% 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 250, 500 and 1000 mg/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, 250, 500 and 1000 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 and ruffled feathers after 24 hours, while birds treated with 500 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 2,4-D technical to the pigeon was calculated to be 565.4 mg/kg bw with the confidence limits (assumed to be 95%) ranging from 377.6-753.3 mg/kg bw. These values are reported in terms of test product. Therefore, the acid equivalent LD50 is 334 mg ae/kg bw.

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

Test System

The acute oral toxicity of 2,4-D Sodium salt (80% WP formulation) 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 1000, 2000 and 4000 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, 1000, 2000 and 4000 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 2000 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 two rates exhibited dullness after 72 h. 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 sodium salt formulation to the chicken was calculated to be 2261.8 mg/kg bw with the confidence limits (assumed to be 95%) ranging from 1510.5-3013 mg/kg bw. The LD50 in acid equivalent terms is 1843 mg ae/kg bw.

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

Test System

The acute oral toxicity of 2,4-D Sodium salt (80% WP formulation) 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 1000, 1500 and 2250 mg/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, 1000, 1500 and 2250 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 1500 mg/kg bw group occurred on day 6, while in the highest treatment group, 1 bird died on day 4, 6 and 7. In this highest treatment group, birds exhibited dullness and ruffled feathers after 24 hours, while birds treated with 1500 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 sodium salt formulation to the pigeon was calculated to be 1611.9 mg/kg bw with the confidence limits (assumed to be 95%) ranging from 1298.7-1925.1 mg/kg bw. The LD50 in acid equivalent terms is 1307 mg ae/kg bw.

In addition to the studies provided to the APVMA for review the US EPA assessed several 2,4-D amine salt 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 A3.33: Additional Acute Avian Toxicity Data Reported in US EPA, 2005

Species	LD50 (mg ac/kg)	LD50 (mg ae/kg)	MRID No.
2,4-D DMA Salt			
Mallard duck *(Anas platyrhynchos)*	>4640	>3851.2	233351
2,4-D DEA Salt			
Northern bobwhite quail *(Colinus virginianus)*	595	404.6	419751-01
2,4-D IPA Salt			
Mallard duck *(Anas platyrhynchos)*	>398	>314.4	00138871
2,4-D TIPA Salt			
Northern bobwhite quail *(Colinus virginianus)*	>405	>218.7	416444-01

No comparable studies for the mallard duck were provided for 2,4-D DEA and TIPA salt, or for the bobwhite quail for 2,4-D IPA salt.

Short-Term

Two avian dietary toxicity studies of a 2,4-D amine salt were provided to the APVMA for review along with two older dietary studies of a formulation. The following results were obtained:

Table A3.34. Summary of Short-Term Bird Toxicity Results for 2,4-D Salts

Test substance	Species	LD50	NOEC	Reference
		(mg ae/kg diet)		
SALTS				
2,4-D DMA	Mallard duck	>4665	466	Long *et al*, 1990a
2,4-D DMA	Bobwhite quail	>4665	2623	Long *et al*, 1990b
2,4-D DMA[1]	Mallard duck	>1899	1899	Fink, 1974a
2,4-D DMA[1]	Bobwhite quail	>1899	879	Fink, 1974b

1) Non-standard test

Test Substance:	**2,4-D DMA**
Report:	Long *et al*, 1990a
Guidelines:	FIFRA Guideline 71-2
GLP:	yes

Test System

The study aimed to evaluate the toxicity of 2,4-D DMA 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 ai. The test consisted of an acclimation period of 8 days, an exposure period of 5 days and a post exposure observation period of 3 days.

The birds were 10 days of age at the initiation of the study. Birds were assigned to 5 test groups and 3 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. The stability of the test substance in the avian diet was studied prior to test initiation. During the test, the average temperature in the brooding compartment of the pens was around 31^oC, average ambient room temperature was around 24^oC and the average relative humidity was around 59%. The photoperiod was 16 hours of light per day during acclimation and through the study.

Following test initiation and continuing until termination, all birds were observed at least twice 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. Average estimated feed consumption was determined for each group for the exposure period (days 0-5) and the observation period (days 6-8). Feed consumption data were reported as an estimate due to unavoidable wastage by the birds.

The LC50 value and 95% CI were calculated by probit analysis, moving average method or the binomial probability method.

Findings

No mortalities at any of the tested concentrations were observed. All birds seemed normal in appearance and behaviour throughout the test period.

The average body weight gain over the 5 day exposure period from the three control groups was 155 g. This compared to 126, 97, 100, 83 and 55 g average body weight gains for the 562, 1000, 1780, 3160 and 5620 ppm test groups respectively. Following the exposure period, weight gains in all groups were relatively similar with 102 g in the control birds compared to 88-101 g increases in body weights in the test groups. In terms of percent of weight gain compared to the control groups during the exposure period, significant reductions were noted in the four highest treatment groups, with weight gains of 81, 63, 64, 53 and 35% of control in the 562, 1000, 1780, 3160 and 5620 ppm test groups respectively. Feed consumption was slightly reduced at the 1780, 3160 and 5620 ppm levels when compared to the controls.

Conclusion

The dietary LC50 value for mallards exposed to 2,4-D DMA in the diet is greater than 5620 ppm (4665 ae ppm). The authors state a NOEC of 562 ppm (468 ae ppm) based on reduction in body weight gain at the 1000 ppm test concentration.

Test Substance:	**2,4-D DMA**
Report:	Long *et al*, 1990b
Guidelines:	FIFRA Guideline 71-2
GLP:	yes
Test System	

The study aimed to evaluate the toxicity of 2,4-D DMA 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 ai. 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 10 days of age at the initiation of the study. Birds were assigned to 5 test groups and 4 control groups. Each treatment and control group contained 10 chicks 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. The test substance in avian diet was studied prior to test initiation. During the test, the average temperature in the brooding compartment of the pens was around $37^{o}C$, average ambient room temperature was around $26^{o}C$ and the average relative humidity was around 56%. The photoperiod was 16 hours of light per day during acclimation and through the study.

Following test initiation and continuing until termination, all birds were observed at least twice 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. Average estimated feed consumption was determined for each group for the exposure period (days 0-5) and the observation period (days 6-8). Feed consumption data were reported as an estimate due to unavoidable wastage by the birds.

The LC50 value and 95% CI were calculated by probit analysis, moving average method or the binomial probability method.

Findings

One bird escaped from the control group and was killed in the recapture attempt. Otherwise, there were no mortalities at any treatment and all birds seemed normal in appearance and behaviour during the study.

Body weight gain was reduced in the highest test group compared to that for the controls. In this 5620 ppm group, average body weight gain was around 40% of that of control birds during the exposure period but comparable during the post exposure observation period. There was no apparent effect on feed consumption at any tested concentration.

Conclusion

The dietary LC50 value for bobwhite quail exposed to 2,4-D DMA in the diet is greater than 5620 ppm (4665 ae ppm). The NOEC was determined to be 3160 ppm (2633 ae ppm) based on the reduction in body weight gain observed at the 5620 ppm test concentration.

Test Substance:	**2,4-D DMA-4**
Report:	Fink, 1974a
Guidelines:	FIFRA Guideline 71-2
GLP:	No

COMMENT: The test substance is stated as DMA-4. This is an old study and there is no other identification of the substance. Other ecotoxicity data for aquatic species

> discussed below identify this product to contain around 49.3% DMA. This is
> assumed to be the case here also.

Test System

The study aimed to evaluate the toxicity of DMA-4 (assumed to be 49.3% 2,4-D
DMA or 40.1% acid equivalent) when administered to juvenile mallard ducks (*Anas
platyrhynchos*) in the diet for 5 days. Nominal test levels were 215, 464, 1000, 2150
and 4640 ppm test substance. In addition, a dieldrin control test was performed with
exposure concentrations of 68, 100, 147, 215 and 316 ppm ac.

Ducklings were hatched and maintained in thermostatically controlled brooders at
37.5^{o}C until 14 days of age. They were then randomly assigned to the negative
control, positive (dieldrin) control and experimental groups with 5 test groups for
each. 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
substances into the diet with corn oil. The birds were exposed to appropriate dietary
concentrations for 5 days and then maintained on a toxicant-free diet for an additional
3 day observation period. The stability of the test substance in the avian diet does not
appear to have been studied prior to test initiation. Environmental conditions of
temperature, humidity and photoperiod during the study are not reported.

Following test initiation and continuing until termination, all birds were observed
daily. Observations of mortality, signs of toxicity and abnormal behaviour were
recorded. Pen body weights were measured at test initiation and termination.
Average estimated feed consumption by pen was determined for each group for the
exposure period (days 0-5). Feed consumption data were reported as an estimate due
to unavoidable wastage by the birds.

Findings

There was no mortality in the negative control groups and the birds appeared normal
for the duration of the study. In the positive (dieldrin) control groups, sub-lethal
effects were recorded at all treatment levels. Mortality of 20, 40, 90, 100 and 100%
were recorded in the 68, 100, 147, 215 and 316 ppm treatment groups respectively.

No mortalities were recorded at any treatment level of 2,4-D DMA. The report notes
that birds in these treatment groups showed no symptoms of toxicity or behavioural
abnormalities at the dosage levels tested.

Conclusion

The dietary LC50 value for mallards exposed to 2,4-D DMA in the diet is greater than
4640 ppm (2288 ppm 2,4-D DMA or 1899 ppm 2,4-D acid). The NOEC is 4640
ppm, the highest rate tested.

Test Substance:	**2,4-D DMA-4**
Report:	Fink, 1974b
Guidelines:	FIFRA Guideline 71-2
GLP:	No

COMMENT: See comment for Fink 1974a above regarding the test substance.

Test System

The study aimed to evaluate the toxicity of DMA-4 (assumed to be 49.3% 2,4-D DMA or 40.9% acid equivalent) when administered to juvenile Bobwhite quail (*Colinus virginianus*) in the diet for 5 days. Nominal test levels were 215, 464, 1000, 2150 and 4640 ppm. In addition, a dieldrin control test was performed with exposure concentrations of 10, 14.7, 21.5, 31.6 and 46.4 ppm ai.

Chicks were hatched and maintained in thermostatically controlled brooders at 37.5^{o}C until 14 days of age. They were then randomly assigned to the negative control, positive (dieldrin) control and experimental groups with 5 test groups for each. Each treatment and control group contained 10 chicks that were not differentiated on sex due to their age. Test diets were prepared by mixing the test substances into the diet with corn oil. The birds were exposed to appropriate dietary concentrations for 5 days and then maintained on a toxicant-free diet for an additional 3 day observation period. The stability of the test substance in the avian diet does not appear to have been studied prior to test initiation. Environmental conditions of temperature, humidity and photoperiod during the study are not reported.

Following test initiation and continuing until termination, all birds were observed daily. Observations of mortality, signs of toxicity and abnormal behaviour were recorded. Pen body weights were measured at test initiation and termination. Average estimated feed consumption by pen was determined for each group for the exposure period (days 0-5). Feed consumption data were reported as an estimate due to unavoidable wastage by the birds.

Findings

There was no mortality in the negative control groups and the birds appeared normal for the duration of the study. In the positive (dieldrin) control groups, sub-lethal effects were recorded at treatment levels of 14.7 ppm and above. Mortality of 10, 20 and 100% were recorded in the 21.5, 31.6 and 46.4 ppm ai ppm treatment groups respectively.

DMA-4 did not cause symptoms of toxicity or behavioural abnormalities at the 215, 464, 1000 or 2150 ppm dosage levels. A 20% mortality, wing droop and lethargy were noted at the 4640 ppm dosage level.

Conclusion

The dietary LC50 value for Bobwhite quail exposed to DMA-4 in the diet is greater than 4640 ppm (2288 ppm 2,4-D DMA or 1899 ppm 2,4-D acid). The NOEC is 2150 ppm.

In addition, the US EPA assessed several 2,4-D amine salt studies for avian dietary toxicity. While no comment can be made on these studies as they have not been assessed by DEH, the results provided by the US EPA are as follows:

Table A3.35: Additional Short-Term 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 DMA Salt			
Northern bobwhite quail *(Colinus virginianus)*	>10,000	>8300	233351
2,4-D DEA Salt			
Northern bobwhite quail *(Colinus virginianus)*	>5620	>3821.6	419751-02
Mallard duck*(Anas platyrhynchos)*	>5620	>3821.6	419751-03
2,4-D IPA Salt			
Northern bobwhite quail *(Colinus virginianus)*	>5620	>4440	00138870
Mallard duck *(Anas platyrhynchos)*	>5620	>4440	00138872
2,4-D TIPA Salt			
Northern bobwhite quail *(Colinus virginianus)*	>5620	>3035	416444-02
Mallard duck *(Anas platyrhynchos)*	>5620	>3035	416444-03

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 DMA, 2,4-D DEA, 2,4-D IPA, 2,4-D TIPA and 2,4-D sodium salt, although only studies for 2,4-D DMA and 2,4-D sodium salt were reviewed. The only standard test was with 2,4-D DMA to bobwhite quail and showed this chemical to be moderately toxic with an LD50 of 415 mg ae/kg bw. The other tests reviewed were non-standard and conducted on the domestic chicken and pigeon. The pigeon was most sensitive to 2,4-D DMA with an LD50 of 334 mg ae/kg bw, but the sodium salt was practically non-toxic to both these non-standard species. Other results where data were not reviewed indicate the 2,4-D DMA is practically non-toxic to the mallard duck and only threshold concentrations were obtained for the IPA and TIPA salts to mallard duck (LD50 >314 mg ae/kg bw) and bobwhite quail (LD50 >219 mg ae/kg bw) respectively.

Four short tem toxicity tests were reviewed for 2,4-D DMA salt, with no defined short term LD50 able to be derived up to 1899 ae ppm for either species on old, non-standard tests or >4655 ae ppm to either species in standard short-term tests. In addition to these reviewed data, seven other results were reported confirming this lack of toxicity through the diet. These results covered the 2,4-D DMA, DEA, IPA and TIPA salts, and indicate that in general, 2,4-D salts should not be toxic to birds when consumed in the diet.

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

Aquatic Toxicity

Fish - Acute

In support of the review of 2,4-D in Australia, several studies were provided for fish acute toxicity with the following results:

Table A3.36. Summary of Acute Fish Toxicity Results for 2,4-D Salts

Test species	System	LC50 (mg ae/L)	Reference
2,4-D DMA			
Fathead minnow (*P. promelas*)	96 h flow-through	186	Alexander *et al*, 1983c
Bluegill sunfish (*L. macrochirus*)	96 h flow-through	293	Alexander *et al*, 1983c
Rainbow trout (*S. gairdneri*)	96 h flow-through	140	Alexander *et al*, 1983c
Rainbow trout (*O. mykiss*)	96 h static	>100	Memmert, 1997a
Tidewater silverside (*M beryllina*)	96 h flow-through	389 (m)	Scott Wart, 1991a
Rainbow trout (*S. gairdneri*)	96 h static	314	Bentley, 1974
Bluegill sunfish (*L. macrochirus*)	96 h static	322	Bentley, 1974
Carp (*C. carpio*)	96 h static	560-1000	Nigitz, 1990a
Rainbow trout (*S. gairdneri*)	96 h static	240	Nigitz, 1990b
Mozambique tilapia (*T mossambica*)	96 h static	35.6[1]	Chittibabu, 2002n
2,4-D Sodium salt			
Mozambique tilapia (*T mossambica*)	96 h static	>80[1]	Chittibabu, 2002o

m = measured concentrations.
  1)   Non-standard test.

Test Material:	**2,4-D DMA (67.3% purity)**
Report:	Alexander *et al*, 1983c
Guidelines:	US EPA Guideline 72-1
GLP:	no

Test system:

The acute toxicity of 2,4-D DMA was tested on three fish species, rainbow trout (*Salmo gairdneri*), bluegill (*Lepomis macrochirus*) and fathead minnow (*Pimephales promelas*) for 96 hours under static conditions. Test vessels were placed in constant-temperature water troughs. Each vessel received 8 litres of dilution water to which 10 fish were indiscriminately added. Gentle aeration proceeded for an acclimation period of at least 4-8 hours prior to addition of the chemical in an extra two litres of dilution water, at which time aeration stopped.

Standard dilution water was taken from Lake Huron. The water was carbon filtered and UV irradiated prior to use. During the study, water quality parameters ranged from pH of 7.8-8.0, hardness of 90-108 mg/L as $CaCO_3$ and alkalinity 77-84 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. The test report states that all concentrations provided are nominal and expressed on a product basis rather than an acid equivalent basis. It is unclear whether concentrations have been corrected for product purity, and is assumed by DEH that this is not the case. Nominal test concentrations for the various species (assumed to be uncorrected for product purity) were:

Species	Exposure concentrations (mg/L)									
Fathead Minnow	0	246	307	384	480	600				
Bluegill	0	100	126	157	197	246	307	384	480	600
Rainbow Trout	0	120	172	245	350	500				

It does not appear any analysis was performed on dilution water to verify exposure concentrations.

The effect criterion was mortality, which was recorded daily. Several statistical methods were used to determine LC50 values. The probit analysis results are reported here.

Findings:

No mortality was recorded in any of the controls used for any of the different species. In the fathead minnow test, dissolved oxygen and pH ranged from 7.0-9.8 mg/L and 6.6-7.9 respectively while temperature was around 17°C. For bluegill, dissolved oxygen and pH ranged from 5.8-9.4 mg/L and 7.3-7.9 respectively while temperature was around 17°C. For rainbow trout, dissolved oxygen and pH ranged from 5.4-9.5 (last 48 hours) mg/L and 7.7-8.1 respectively while temperature was around 12°C.

Mortality figures for the three species were reported as follows:

Table A3.37: Mortality Results for Fathead Minnow, Bluegill and Rainbow Trout

Fathead minnow		Bluegill		Rainbow trout	
mg/L	%mortality	mg/L	%mortality	mg/L	%mortality
0	0	0	0	0	0
246	10	100	0	120	0
307	40	126	0	172	10
384	90	157	0	245	30
480	70	197	0	350	100
600	100	246	10	500	100
		307	10		
		384	20		
		480	20		
		600	80		

Conclusion:

The LC50s calculated by probit analysis for the test substance with corresponding 95% confidence intervals are shown below. LC50 values have also been corrected for purity, and shown in acid equivalent terms.

	LC50 mg product/L	95% CI (mg/L)	LC50 mg ac/L	LC50 mg ae/L
Fathead Minnow	334	281-382	225	186
Bluegill	524	441-758	353	293
Rainbow Trout	250	215-293	168	140

Test Material:	**Herbizid Marks D** (2,4-D DMA, 514 g ae/L solution)
Report:	Memmert, 1997a
Guidelines:	OECD TG 203
GLP:	yes

Test system:

The acute toxicity of the formulation Herbizid Marks D (containing 2,4-D at 514 g/L in its DMA form) was tested in a limit test on rainbow trout (*Oncorhynchus mykiss*) under static conditions for 96 hours. At the start of the study, 10 fish from the

acclimated test fish batch were measured and showed an average length of 4.9 cm and average body weight of 1.3 g.

One glass aquarium with 15 L test medium was used for each treatment, namely the control and a single treatment rate of a nominal 200 mg/L formulation (around 100 mg/L 2,4-D DMA). At the start of the test, 7 fish were introduced into each aquarium. The test media were slightly aerated during the test. Water temperature was around 14°C and a 16 h light photoperiod was maintained. Water hardness was around 250 mg/L as $CaCO_3$.

The test fish were observed after around 4, 24, 48, 72 and 96 hours for symptoms of intoxication and mortality. Water quality parameters were measured before the start of the test then daily. To determine the stability of the test compound, water samples were taken at days 0 and 4 and the concentrations of the active ingredient, 2,4-D, analysed.

Findings:

The test medium was noted as being clear throughout the study. The pH values ranged from 7.6-7.9 while dissolved oxygen ranged from 7.9-9.3 mg/L in both the control and test water. Water temperature was a constant 14°C throughout the test.

Analytically determined test substance concentrations amounted to 97% of the nominal value during the test.

No mortality or signs of intoxication were observed at any time throughout the test.

Conclusions:

The 96 h NOEC was the highest concentration tested, 200 mg/L formulation, equating to around 100 mg ae/L.

Test Material:	2,4-D DMA (66.8% purity)
Report:	Scott Ward, 1991a
Guidelines:	US EPA FIFRA Guideline 72-3
GLP:	yes

Test system:

The acute toxicity of 2,4-D DMA salt was tested on tidewater silverside (*Menidia beryllina*) under flow-through conditions for 96 hours. Measured concentrations tested were control, 224, 357, 590, 1020 and 1657 mg/L 2,4-D DMA and were based on the results of a preliminary study. 20 fish per concentration (not replicated) were used in the experiment. Dilution water consisted of filtered sea water diluted to a salinity of 22 ppt which was vigorously aerated prior to use. Flow-through conditions were such that there were 7 daily volume additions to eacmh exposure chamber.

The test was conducted using 24 L glass aquaria with 15 L dilution water. For 7 days prior to test initiations, fish were maintained in saltwater with salinity of 23-25 ppt and temperature of 19.5-24.1°C. During the 48 h period prior to testing, fish were held under test conditions and there were no mortalities. Fish for this test ranged from 26-32 mm in length and weighed 0.11-0.28 g wet weight. The photoperiod consisted of 16 h light per day.

All aquaria were examined daily for mortality and behavioural changes. Dead fish were removed. Water quality parameters were measured daily in each exposure vessel until test termination or 100% mortality. Exposure concentrations were verified through chemical analyses with water samples collected from each exposure group and the control on days 0, 2 and 4.

The LC50s and confidence limits were calculated statistically using various methods throughout the test. The 96 h results were calculated using probit analysis.

Findings:

Measured concentrations are used to report results. The test temperature ranged from 21-5-22.7°C and salinity from 21-24 ppt during the test. Dissolved oxygen concentrations remained at $\geq$51% saturation in all test containers during the study, and $\geq$84% saturation in the control water and the two highest concentrations in which fish died quickly. The dissolved oxygen concentrations were lower in the three lower test concentrations. The author observed that the presence of 2,4-D DMA salt was stressful to the fish and resulted in a higher rate of respiration, which was manifested in lower dissolved oxygen concentrations. The lowest dissolved oxygen concentrations occurred in the lowest test concentration, which had the greatest survival of fish. The pH ranged from 8.2-8.5 in all test containers during the test.

The following mortality was observed (measured concentrations reported):

Table A3.38: Cumulative Mortality to Tidewater Silverside

Measured concentration (mg/L)	Cumulative mortality (%)			
	24 h	48 h	72 h	96 h
0	5	5	5	10
224	0	0	0	5
357	0	0	0	20
590	5	20	55	70
1020	80	100	100	100
1657	200	100	100	100

The main sub-lethal effect observed was darker colouration of fish. In the lowest test group, 1 fish was observed as being dark at 96 h, with four darkened fish observed at 72 h in the 357 mg/L group. Discolouration was first noticed at 24 h in the 590 mg/L group. While all fish had died at 48 h in the 1020 mg/L group, lethargy and gulping were observed in two of the four surviving fish at 24 hours.

Conclusions:

The 96 h LC50 for 2,4-D using tidewater silverside was determined to be 469 mg/L (measured) with 95% confidence limits of 403-547 ppm. In acid equivalent terms, this equates to around 389 mg ae/L. The author states a NOEC could not be determined due to mortality and discolouration observed at the lowest concentration tested. However, the mortality in this group was still less than the control mortality. Discolouration was observed in 1 fish only. The NOEC may be expected to approximate the lowest test concentration of 224 mg/L (186 mg ae/L).

Test Material:	**DMA-4 (49.3% 2,4-D DMA)**
Report:	Bentley, 1974
Guidelines:	US EPA Guideline 72-1
GLP:	no

Test system:

The acute toxicity of the formulation DMA-4 was tested on bluegill (*Lepomis macrochirus*) and rainbow trout (*Salmo gairdneri*) for 96 hours under static conditions. Bluegill had a mean weight of 1.0 g and a mean length of 37 mm while rainbow trout had a mean weight and length of 1.3 g and 62 mm respectively. Test animals were held in laboratory hatchery facilities for at least 30 days prior to testing, where well water had a temperature of around $20^{o}C$ (apparently for both species, although this would appear too warm for rainbow trout), hardness of 47 mg/L as $CaCO_3$ and pH of 7.1. The static bioassays were conducted in 19.6 L vessels kept in water baths at $20^{o}C$ for bluegill and $10^{o}C$ for rainbow trout. The fish were acclimated over a 48 h period prior to test conditions of temperature and water quality. Based on results of a range finding test, the definitive test consisted of five concentrations, being 0, 210, 280, 370, 490 and 650 mg/L for bluegill and 0, 210, 280, 320, 370, 490, 650 and 870 mg/L for rainbow trout. This is an old study and the test material is not well characterised. The results (and consequently the above exposure concentrations) are in terms of active constituent, not product. It is unclear as to whether this then refers to 2,4-D acid or 2,4-D DMA. In the absence of guidance from the test report, it will be assumed this is 2,4-D DMA salt.

Fish were introduced to the test vessel within 30 minutes after the compound was added. Ten fish were exposed to each concentration. It appears only one replicate per concentration was used.

The reconstituted water used in the test system was prepared by adding 48 mg $NaHCO_3$, 30 mg $CaSO_4$, 30 mg $MgSO_4$ and 2 mg KCl per litre of deionised water. The pH of the standard diluent was 7.1 and the total hardness was 35 ppm as $CaCO_3$. Dissolved oxygen ranged from 8.9 initially to 4.3 (difficult to read in test report) mg/L at the end of the tests.

LD50 (defined in the test as a median tolerance limit – TL50, but still the nominal concentration of the test compound causing 50% mortality) and 95% CI's were calculated using probit analysis and least squares regression analysis.

Findings:

The report notes that fish generally became dark and lethargic, lost equilibrium, and expired. However, it makes no comment as to the concentration levels or time during the test as to when sub-lethal effects were noted. The mortality results are summarised as follows:

Table A3.39: Mortality to Bluegill and Rainbow Trout

Bluegill				Rainbow Trout			
% Mortality Observed				% Mortality Observed			
Exposure (mg/L)	24 h	48 h	96 h	Exposure (mg/L)	24 h	48 h	96 h
0	0	0	0	0	0	0	0
210	0	0	0	210	0	0	0
280	0	0	0	280	0	0	10
370	0	10	10	320	0	0	20
490	70	80	100	370	0	0	10
650	100	100	100	490	50	50	90
				650	100	100	100
				870	100	100	100

Conclusion:

The 96 h LD50 of 2,4-D DMA to bluegill was calculated to be 387 mg ac/L (95% CI 279-509 mg/L) while that to rainbow trout was calculated to be 377 mg ac/L (95% CI 324-440 mg/L). The corresponding LD50s for bluegill and rainbow trout in terms of acid equivalence would be around 322 and 314 mg ae/L.

Test Material:	**2,4-D DMA**
Report:	Nigitz, 1990a
Guidelines:	OECD Guideline 203; EEC Guideline C-1
GLP:	Yes

Test system:

The acute toxicity of 2,4-D as the DMA salt was evaluated to carp (*Cyprinus carpio*) over 96 hours in a static system. Carp were an average 3.1 cm in length and 0.43 g in weight. They were acclimated in 100 L tanks for at least 14 days. Test vessels were 10 L in capacity and the test medium was tap-water which was continuously aerated. 10 fish per concentration were used at a loading of 1 g fish per litre of test medium (10 fish per 4 L test medium). A photoperiod of 16 hours per day was maintained and the room temperature was 21.5-24°C. Fish were not fed from 24 hours prior to the test and during the total test period.

Based on the results of preliminary testing, nominal concentrations used in the definitive study were 0, 100, 180, 320, 560 and 1000 mg ae/L. Nominal concentrations are expressed as weight of 2,4-D Acid (saturated with DMA) added to the test media. Chemical analysis was performed during the main study to verify actual concentrations.

Mortality and sub-lethal effects were recorded at 4, 24, 48, 72 and 96 hours after the start of exposure. Dissolved oxygen and pH were measured in all vessels prior to addition of the fish, and daily thereafter. Water temperature was measured in the blank control vessels every day of the test.

Findings:

The pH ranged from 7.5-8.5, with 8.6 recorded at 100 and 180 mg/L from 72 hours of exposure and in the control at the end of the study. Oxygen concentration in the test media was found to be >5 mg/L for all measurements performed during the main

study. The temperature of the test medium measured in the blank control varied from 21.5-22°C.

Analysis of water samples at the start of the main test revealed mean actual concentrations (2,4-D Acid) of 106, 98 and 98% relative to the nominal concentrations of 100, 320 and 1000 mg/L respectively. During the exposure period, the actual concentration remained >80% at all three concentrations sampled.

At 48 hours of exposure, all fish had died at 1000 mg/L. At the end of the study, 10% mortality was recorded at 560 mg/L whereas no mortality was observed at the other concentrations or the controls. Effects other than mortality, were observed at 320 mg/L and higher. From 48 and 72 hours to the end of the exposure all fish at 560 mg/L and 320 mg/L respectively, were hypoactive as compared to the fish in the control group.

Conclusion:

Based on these data, the LC50 at 96 hours appears to be between 560 and 1000 mg ae/L. The NOEC was 180 mg ae/L.

Test Material:	**2,4-D DMA**
Report:	Nigitz, 1990b
Guidelines:	OECD Guideline 203; EEC Guideline C-1
GLP:	Yes

Test system:

The acute toxicity of 2,4-D as the DMA salt was evaluated to rainbow trout (*Salmo gairdneri*) over 96 hours in a flow-through system. Fish were an average 6.8 cm in length and 2.77 g in weight. They were acclimated in 200 L tanks for at least 14 days. Test vessels were 30 L in capacity and the test medium was tap-water which was continuously aerated. The flow rate through the test was 6 litres per hour. 10 fish per concentration were used at a loading of 0.9 g fish per litre of test medium (10 fish per 30 L test medium). A photoperiod of 16 hours per day was maintained and the room temperature was 12.5-16°C. Fish were inadvertently fed at day 1 and day 2.

Based on the results of preliminary testing, nominal concentrations used in the definitive study were 0, 100, 180, 320, 560 and 1000 mg ae/L. Nominal concentrations are expressed as weight of 2,4-D Acid (saturated with DMA) added to the test media. Chemical analysis was performed during the main study to verify actual concentrations.

Mortality and sub-lethal effects were recorded at 1, 24, 48, 72 and 96 hours after the start of exposure. Dissolved oxygen and pH were measured in all vessels prior to addition of the fish, on nominal day 2 and 4 and directly after recording 100% mortality. Water temperature was measured in the blank control vessels every day of the test.

Findings:

The pH ranged from 8.0-8.4. Oxygen concentration in the test media was found to be >5 mg/L for all measurements performed during the main study. The temperature of the test medium measured in the blank control varied from 14.5-15.5^{o}C.

In general concentration analysis of the samples taken at the start of the main test revealed that the actual test concentrations were in agreement with the nominal concentrations except at 100 mg/L where the measured concentration was 67 mg/L (as 2,4-D acid). Analysis of samples taken at 21 hours after introduction of the fish resulted in a mean actual test concentration of 95 mg/L. Since the actual concentrations in general were >80% of the nominal concentrations, nominal values (as acid equivalents) are used for reporting.

At 560 mg/L and higher, all ten fish had died after 24 hours whereas at 320 mg/L nine of the ten fish had died. At the end of the study, 100% mortality was recorded at 320 mg/L and higher, whereas at and below 180 mg/L no mortality was seen. No comments on sub-lethal effects are made except that from 48 h onwards in the 180 mg/L group, one or two of the fish were noted as discoloured.

Conclusion:

All mortality occurred between the tested concentrations of 180 and 360 mg ae/L (no concentrations tested between these two). Consequently, the LC50 is reported as 240 mg ae/L (95% CI 180-320). Based on discolouration effects at 180 mg ae/L, the NOEC is stated to be 100 mg ae/L.

Test Substance:	**2,4-D DMA SL Salt**
Report:	Chittibabu, 2002n
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 DMA salt (59.1% w/w 2,4-D) was performed on the freshwater fish, Mozambique tilapia (*Tilapia mossambica*) over 96 hours in a static test. 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 25, 35, 49, 69 and 97 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 parameter, pH was 7.1, temperature was 22°C, dissolved oxygen was 7.8 mg/L and total hardness as $CaCO_3$ was 323 mg/L.

Cumulative mortality in the 25, 35, 49, 69 and 97 mg product/L groups after 96 hours was 0, 10, 30, 60 and 90% with no mortality observed in the control fish. Fish exposed to the highest test concentration were observed to exhibit a loss of equilibrium and random opercular movement.

Conclusion:

The 96 h LC50 for the Mozambique tilapia is 60.3 mg product/L with confidence limits (assumed to be 95%) of 54.6-65.9 mg/L. These values are expressed in the report as mg/L of 2,4-D DMA SL Salt, which was shown to have a 2,4-D content of 59.1%. The resulting acid equivalent value would be an LC50 of 35.6 mg ae/L.

Test Substance:	**2,4-D Sodium salt**
Report:	Chittibabu, 2002o
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 Sodium salt (80% WP formulation) 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 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 25, 50, 75 and 100 mg product/L (nominal) along with a water control group. Observations for mortality and abnormal behaviour was made at 3 and 6 h, and thereafter, every 24 h until 96 h.

Findings:

At the time of measuring water quality parameter, pH was 7.1, temperature was 22°C, dissolved oxygen was 7.8 mg/L and total hardness as $CaCO_3$ was 238 mg/L.

No mortality or behavioural abnormalities were observed throughout the test.

Conclusion:

The 96 h LC50 for the Mozambique tilapia is >100 mg product/L (>80 mg ae/L). The NOEC was the highest level tested of 100 mg product/L (80 mg ae/L).

In addition, the US EPA reviewed several studies for the 2,4-D sodium salt, 2,4-D DMA, 2,4-D DEA, 2,4-D IPA and 2,4-D TIPA 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:

Freshwater acute fish toxicity – amine salts and sodium salt. Results are in measured concentrations unless otherwise indicated.

Table A3.40: Additional Acute Freshwater Fish Toxicity Data Reported in US EPA, 2005

Species	Test system	LC50 (mg ac/L)	LC50 (mg ae/L)	MRID
2,4-D Sodium Salt				
Rainbow trout *(Oncorhynchus mykiss)*	96 h Static	>100 (nominal)	>91 (nominal)	53986
2,4-D DMA Salt				
Rainbow trout *(Oncorhynchus mykiss)*	96 h	>1000	>830	233350
Bluegill Sunfish *(L. macrochirus)*	96 h	>121	>100	419751-04
Bluegill Sunfish *(L. macrochirus)*	96 h	>1000	>830	234027
Fathead minnow *(P. promelas*	96 h	318	264	419715-04
2,4-D Diethanolamine (DEA) Salt				
Rainbow trout *(Oncorhynchus mykiss)*	96 h Static	>120	>81.6	419751-04
Bluegill Sunfish *(L. macrochirus)*	96 h, assumed static	>121	>82.3	419751-04
Fathead minnow *(P. promelas*	96 h, assumed static	344	234	419751-04
Bluegill Sunfish *(L. macrochirus)*	96 h, assumed static	149	101	0073-091-01
2,4-D IPA				
Rainbow trout *(Oncorhynchus mykiss)*	96 h, assumed static	2840	2244	01338869
Bluegill Sunfish *(L. macrochirus)*	96 h, assumed static	1700	1343	01338869
Fathead minnow *(P. promelas*	96 h, assumed static	2180	1722	01338869
2,4-D TIPA				
Rainbow trout *(Oncorhynchus mykiss)*	96 h, assumed static	300	162	413538-03
Bluegill Sunfish *(L. macrochirus)*	96 h, assumed static	401	217	413538-03

Table A3.41: Additional Acute Estuarine/Marine Fish Toxicity Data Reported in US EPA, 2005

Species	Test system	LC50 (mg ac/L)	LC50 (mg ae/L)	MRID
2,4-D DMA salt				
Sheepshead minnow *(C. variegates)*	96 h	>560	465	233351
2,4-D Diethanolamine (DEA) Salt				
Tidewater silverside *(Menidia beryllina)*	96 h, assumed static	>118	>80.24	420183-01
2,4-D IPA				
Tidewater silverside *(Menidia beryllina)*	96 h, assumed static	237	187	414290-01
2,4-D TIPA				
Tidewater silverside *(Menidia beryllina)*	96 h, assumed static	376	203	414290-04

No data on estuarine/marine species were submitted on the 2,4-D sodium salt. However, since the environmental fate data indicates that the salts and amines of 2,4-D rapidly degrade to the acid equivalent, the data from the 2,4-D acid may be used to characterize the risk to marine fish. No data will be required at this time.

NOTE: for the US EPA, the preferred test species is the sheepshead minnow, however, due to the low toxicity of the tidewater silverside, their guideline (72-3a) was considered fulfilled.

Fish – Sub-chronic/Chronic

Two tests were submitted to the APVMA with the following results:

Table A3.42. Summary of Chronic/Sub-Chronic Fish Toxicity Results (Flow through conditions)

Test species	Test system	NOEC/MATC (mg ae/L)	Reference
2,4-D DMA			
Rainbow trout (*O. mykiss*)	28 d	NOEC = 100	Monk, 1990
Fathead minnow (*P. promelas*)	31 day ELS	14.2/18.3 (m)	Dill *et al*, 1990

Test Material:	**2,4-D DMA (formulation, 500 g/L 2,4-D)**
Report:	Monk, 1990
Guidelines:	OECD Guideline 204
GLP:	Yes

Test system:

OECD TG 204 is a 14 day prolonged fish study. This study was extended to 28 days.

The sub-lethal toxicity of 2,4-D as the DMA salt was evaluated to rainbow trout (*Oncorhynchus mykiss*) in a flow-through system for 28 days. The test substance was U 46 D-Fluid, an aqueous formulation of 2,4-D DMA salt (500 g/L 2,4-D). Rainbow trout were 69 days old at the start of the test and were an average 5 cm in length and 1.24 g in weight. They were acclimated to test conditions for 18 days prior to test initiation. The photoperiod was 16 h light per day. Test concentrations (in terms of acid equivalent) were 0, 6.25, 12.5, 25, 50 and 100 mg/L

The test was carried out in a continuous flow-through system with aquaria maintaining a constant water volume of around 60 L. The flow rate was 10 L/h and aeration was continuous. The test temperature was around 15°C throughout the study. Dilution water had a pH around 7.8 and an oxygen content >80% of maximum saturation. 20 fish were used per test group. 2 days prior to the beginning of the study, a batch of test fish of approximately uniform weight was established.

Mortality was determined generally daily, while clinical signs were assessed at least on workdays. Body weights and length were determined at test initiation and termination.

Aquarium water was analysed for 2,4-D concentrations on days 0, 7, 14, 21 and 28. Statistical evaluation of body weight and length was performed by one-way analysis followed by Dunnett's test.

Findings:

Analysis of water concentrations showed measured concentrations in good agreement with nominal ranging from 94%-108% over the course of the study. Nominal concentrations are therefore used for reporting. The pH remained relatively constant throughout the study, ranging from 7.7-8.0 while dissolved oxygen ranged from 90-100% maximum saturation.

No compound-related mortalities occurred, with only one death noted in the control group of fish on day 18 of the study. No toxic signs were observed in any of the test groups including the control.

Table A3.43: Body Weight and Length Results

Exposure (mg/L)	Mean body weights (g)		Mean body lengths (mm)	
	Day 0	Day 28	Day 0	Day 28
0	1.25	3.79	5.00	7.13
6.25	1.25	3.86	5.03	7.18
12.5	1.24	3.84	5.01	7.16
25	1.26	3.87	4.99	7.15
50	1.24	3.92	4.95	7.14
100	1.22	3.43	4.99	6.76

None of the body weights or lengths are considered significantly different from the control group. Body weights at the end of the study were all greater than the control group except the 100 mg/L group where they were 9.5% lower. Similarly, body lengths were reduced in the 100 mg/L group and were around 5.2% lower than the control group. This compared to slightly longer lengths in all other exposure groups when compared to control fish lengths.

Conclusion:

The threshold level for lethal effects of 2,4-D was determined to be >100 mg ae/L. The threshold for sub-lethal effects of 2,4-D was determined to be 100 mg ae/L.

Test Material:	2,4-D DMA salt (66.8% purity)
Report:	Dill *et al*, 1990
Guidelines:	US EPA Guideline 72-4
GLP:	yes

Test system:

The study was undertaken to determine the chronic toxicity of 2,4-D to fathead minnow (*Pimephales promelas*) embryos and larvae during an early life stage test with continuous aqueous exposure over 31 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), 17.1, 28.4, 46.3, 77.4 and 120.7 mg/L 2,4-D DMA.

An intermittent flow proportional diluter was used for the test set to deliver at least 15 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 28 days post day-to-mean hatch of the controls (31 days total). At test termination, all surviving fish were measured for weight and length.

Dissolved oxygen, temperature and pH were recorded from each replicate 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. 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 25.3-25.8°C throughout the study. Dissolved oxygen concentrations ranged from 6.6-9.1 mg/L in all vessels throughout the study. The pH ranged from 7.4-7.8 in all vessels throughout the study.

Biological results are summarised in Table A3.41 below.

Table A3.44: ELS toxicity (31 d) of 2,4-D DMA on Fathead minnow

Concentration [mg ac/L]	Control	17.1	28.4	46.3	77.4	120.7
Embryos hatched (%)	100	98.8	98.8	97.5	97.5	98.7
Normal Larvae at Hatch (%)	98.8	97.4	100.0	93.7	94.9	97.5
28 day Larval Survival (%)	97.5	90.0	85.0*	37.5*	2.5*	0*
Mean Wet Weight (mg)	59.1	57.5	50.4	43.0*	67.0*[a]	0*
Mean Length (mm)	15.6	14.8	13.9*	12.6*	15.0*[a]	0*

* - Statistically different from the control. a) based on only two survivors.

No statistical difference was demonstrated between the treated groups and the control group for the percent hatched and percent normal at hatch. However, average weight was significantly reduced at 46.3 mg/L while average length was significantly reduced at 28.4 and 46.3 mg/L. On day 7 post hatch, larval survival was statistically decreased at the two highest concentrations, while at 28 days, it was significantly reduced at all but the lowest concentration tested.

Conclusion

The NOEC was established to be the lowest concentration tested of 17.1 mg/L (14.2 mg ae/L). The MATC was calculated to be 22 mg/L (18.3 mg ae/L).

In addition to those studies provided to the APVMA, the US EPA reviewed the following early life stage test of the 2,4-D DEA salt.

Table A3.45: Additional Freshwater Fish ELS Data (Flow-Through) Reported in US EPA, 2005. Results are in measured concentrations unless otherwise indicated.

Species	NOEC/LOEC (mg ae/L)	MATC (mg ae/L)	Endpoints affected	MRID
2,4-D Diethanolamine (DEA) Salt				
Fathead Minnow (*Pimephales promelas*)	19.8/66.6	36.3	Larval survival	420183-04

No chronic data were submitted on estuarine or marine species.

Amphibians

Test Material:	**2,4-D DMA (67.3% pure)**
Report:	Palmer and Krueger, 1997c
Guidelines:	Full protocol provided
GLP:	Yes

Test system:

Acute toxicity of 2,4-D DMA 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), 87.5, 145, 242, 404 and 673 mg ac/L. Mean measured test concentrations were determined from samples collected at the beginning and end of the test.

Tadpoles were held for around 14 days and acclimated to test conditions for around 51 hours prior to test initiation. During the 14 day holding period, water temperatures ranged from 21-21.8^{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 50 mg. Loading was calculated to be 0.23 g tadpole/L.

Test chambers were 2 L glass beakers containing around 1.8 L test solution with a water depth around 15.5 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 or until 100% mortality. Hardness and alkalinity were measured in the highest exposure group and control group at test initiation.

Organisms were observed for mortality and behaviour at 4, 24, 48, 72 and 96 hours. The 96 h LC50 was calculated by the binomial method.

Findings:

Mean measured concentrations ranged from 101-102% nominal, and in terms of 2,4-D acid, the measured exposure concentrations were 69.8, 117.5, 194.5, 320.5 and 535.5 mg ae/L. Temperature ranged from 21.5-23.0^{o}C and dissolved oxygen from 8.6-8.8 mg/L at the start of the study to 5.6-6.1 mg/L at the end of the study. 2,4-D concentrations did not appear to impact pH in this study with a range of 8.1-8.4 in all concentrations and the control throughout the test. At the start of the test, hardness and alkalinity were 136 and 180 mg/L as $CaCO_3$ respectively in the dilution water.

Mortality data were reported as follows:

Table A3.46: Cumulative Mortality to Tadpoles

Exposure (mg ae/L)	Cumulative mortality (%)			
	24 h	48 h	72 h	96 h
0	0	0	0	0
69.8	0	0	0	0
117.5	0	0	0	5
194.5	5	15	45	60
320.5	0	80	100	100
535.5	30	100	100	100

All surviving tadpoles were recorded as being normal in appearance throughout the test.

Conclusion:

The 96 h LC50 for leopard frog tadpoles exposed to 2,4-D as the DMA salt was calculated to be 337 mg test material/L. Correcting this for purity, the 96 h LC50 was around 227 mg ac/L, or as the acid equivalent, around 188 mg ae/L. The 95% CI range was 166-213 mg ae/L. Based on the mortality and observation data, the NOEC was determined to be 69.8 mg ae/L.

Aquatic Invertebrates - Acute

The APVMA received six studies addressing this end-point with the following results:

Table A3.47. Summary of Acute Aquatic Invertebrate Toxicity Results for 2,4-D Salts

Test species	System	LC50 (mg ae/L)	Reference
2,4-D DMA			
Daphnia magna	48 h static	103	Alexander *et al*, 1983c
Daphnia magna	48 h static	NOEC = 102	Memmert, 1997b
Pink shrimp (*Panaeus duorarum*)	96 h flow through	150 (m)	Scott Ward, 1991b
Eastern oyster (*C. virginica*)	96 h flow through	113 (m)	Scott Ward, 1991c
Eastern oyster (*C. virginica*)	96 h flow through	EC50 = 85-131	Heitmuller, 1975
Fiddler crabs (*Uca pugilator*)	96 h flow through	>410.8	Heitmuller, 1975
Pink shrimp (*Panaeus duorarum*)	96 h flow-through	>410.8	Heitmuller, 1975

m = measured concentrations.

While one of the generally more sensitive species, mysid shrimp, was not tested DEH considers there are sufficient data available to fulfil this end-point.

Test Material:	**2,4-D DMA (67.3% purity)**
Report:	Alexander *et al*, 1983c
Guidelines:	US EPA Guideline 72-2
GLP:	no

Test system:

The acute toxicity of 2,4-D DMA 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°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 7.9, hardness of 144 mg/L as $CaCO_3$ and alkalinity 57 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°C. Exposure concentrations were control, 64.8, 100, 180, 300 and 500 mg/L with 10 daphnids exposed to each group in triplicate (30 animals total per group). The test report states that all concentrations provided are nominal and expressed on a product basis rather than an acid equivalent basis. It is unclear whether concentrations have been corrected for product purity, and is assumed by DEH that this is not the case.

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

Findings:

There was no mortality in the control group throughout the study, and the oxygen concentration in the test beakers did not fall below 8.9 mg/L while the pH remained between 8.0-8.2. Mortality findings for the controls and test concentrations are as follows:

Table A3.48: Mortality Results for *Daphnia magna*

Concentration (mg/L)	% Mortality	
	24 hours	48 hours
Control	0	0
64.8	0	10
100	0	20
180	0	37
300	24	80
500	70	97

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

Conclusion:

The 48 h LC50 was calculated by probit analysis to be 184 mg/L (95% CI 156-271 mg/L). This is on a product basis, not an acid equivalent. This product is noted as being only 67.3% pure which would make the LC50 around 124 mg 2,4-D DMA/L, or around 103 mg ae/L.

Test Material:	**Herbizid Marks D** (2,4-D 514 g/L solution in its DMA form)
Report:	Memmert, 1997b
Guidelines:	OECD TG 202
GLP:	yes

Test System:

A 48 h toxicity test was conducted to evaluate the influence of the formulation Herbizid Marks D on the mobility of *Daphnia magna*. The test report states the active ingredient is 2,4 D, but the chemical name is 2,4-dichlorophenoxyacetic acid, dimethylamine salt. At the start of the test, daphnids were between 6-24 hours old.

In each treatment, the single test concentration and the control, 20 daphnids were tested in two replicates of 10 each. Water temperature was 20-21°C and a 16 h light to 8 h dark photoperiod was maintained. Dilution water had hardness of 250 and alkalinity of 80 mg/L as $CaCO_3$ respectively.

The only tested concentration was a nominal 200 mg product (100 mg ae)/L. The test medium was prepared by dissolving 60 mg of the product in 300 mL water with intense stirring.

Immobility was determined after 24 and 48 hours. At the start and end of the test, the pH and dissolved oxygen concentrations were determined in the test medium and the control. The water temperature was determined in one control beaker at the start and the end of the test. The actual exposure concentration of 2,4-D was analysed in one of each duplicate test media samples at 0 and 48 hours.

Findings:

The analytically determined test substance concentrations in the test medium ranged from 98-101% of the nominal value during the test period.

Dissolved oxygen concentrations in the test media were at least 8.0 mg/L or higher and pH values ranged from 8.0-8.1. No remarkable observations were made concerning the appearance of the test substance in the test medium.

In the control and test concentration, no animal was immobilised or dead during the 48 hour test period.

Conclusion:

The 48 h NOEC was determined to be at least 200 mg product/L (102 mg ae/L).

Test Material:	2,4-D DMA (66.8% purity)
Report:	Scott Ward, 1991b
Guidelines:	US EPA FIFRA Guideline 72-3
GLP:	yes

Test system:

The acute toxicity of 2,4-D DMA salt was tested on pink shrimp (*Penaeus duorarum*) under flow-through conditions for 96 hours. The shrimp were maintained in the laboratory for at least 17 days following collection. During the 48 h prior to test initiation, they were maintained in natural seawater with salinity of 22-23 ppt and a temperature of 23.1-27.1^{o}C. Young adult shrimp used for this test ranged from 42-62 mm length and 0.65-1.85 g weight. Nominal concentrations tested were control, 35, 58, 96, 161 and 268 mg/L 2,4-D DMA and were based on the results of a preliminary study. 20 shrimp per concentration (not replicated) were used in the experiment. Dilution water consisted of filtered seawater diluted to a salinity of 20 ppt that was vigorously aerated prior to use. Flow-through conditions were such that there were 5 daily volume additions to each exposure chamber.

The test was conducted using 24 L glass aquaria with 15 L dilution water. The photoperiod consisted of 16 h light per day.

All aquaria were examined daily for mortality and behavioural changes. Dead shrimp were removed. Water quality parameters were measured daily in each exposure vessel until test termination or 100% mortality. Exposure concentrations were verified through chemical analyses with water samples collected from each exposure group and the control on days 0, 2 and 4.

The LC50s and confidence limits were calculated statistically using various methods throughout the test. The 96 h results were calculated using the moving average method.

Findings:

Mean measured concentrations ranged from 106-119% of nominal values and are used to report results. The test temperature ranged from 20.7-22.6^{o}C and salinity from 21-24 ppt during the test. Dissolved oxygen concentrations ranged from 4.7-6.7 mg/L in the control vessel. In all exposure vessels, dissolved oxygen fell to less than 60% saturation, generally by day 3 of the study. This is a deviation from the study

protocol, and is theorised by the author as being due to an increased respiratory rate from exposed shrimp. 2,4-D concentrations did not affect pH with pH ranging from 8.2-8.5 in all exposure vessels and the control throughout the study.

The following mortality was observed (measured concentrations reported):

Table A3.49: Cumulative Mortality Results for Pink Shrimp

Measured concentration (mg ac/L)	Cumulative mortality (%)			
	24 h	48 h	72 h	96 h
0	0	5	5	5
39	0	0	5	5
65	0	0	0	0
114	5	5	15	20
170	0	10	20	25
287	15	55	80	100

After 96 hours exposure, the authors state that no abnormal physical or behavioural effects were noted. However, if an increased respiration rate was responsible for the drop in dissolved oxygen, this should be regarded as a sub-lethal effect from 2,4-D exposure. It should be noted that the reduction in dissolved oxygen was not particularly dose responsive with saturation in the 39, 65, 114, 170 and 287 mg ac/L groups being about 55, 52, 40, 39 and 57% saturation respectively at day 4 compared to around 71% saturation in the control.

Conclusions:

The 96 h LC50 for 2,4-D DMA exposure to pink shrimp was determined to be 181 mg/L (measured) with 95% confidence limits of 159-207 ppm. In acid equivalent terms, this equates to around 150 mg ae/L. The author states a NOEC is 65 mg/L (54 mg ae/L) based on no mortality or observed sub-lethal effects at this level. However, they clearly state in their report that dissolved oxygen in the control vessels were >60% saturation during the 96 h test while in all test concentrations it was below 60% saturation. The lowering of dissolved oxygen was generally noted on day 3 and the authors state this appears to result from an increased respiratory rate of exposed shrimp.

DEH considers this as a sub-lethal effect from exposure to the chemical and as such, the NOEC should be stated as <39 mg ac/L or >32.5 mg ae/L.

Test Material:	2,4-D DMA (66.8% purity)
Report:	Scott Ward, 1991c
Guidelines:	US EPA FIFRA Guideline 72-3
GLP:	yes

Test system:

The acute toxicity of 2,4-D DMA salt was tested by measurement of shell deposition in the Eastern oyster, (*Crassostrea virginica*) under flow-through conditions for 96 hours. The oysters were maintained in the laboratory for 16 days following collection. They were maintained in natural seawater with salinity of 31-33 ppt and a temperature of 18.1-23.3°C. Oysters for this test ranged from 24.4-34.7 mm length (umbo to distal valve edge) and 0.39-1.29 g weight. Nominal concentrations tested were control, 23, 39, 65, 108, 180 and 300 mg/L 2,4-D DMA and were based on the results of a preliminary study. 20 oysters per concentration (not replicated) were used

in the experiment with between 2-5 mm of shell removed from the margin of each oyster. Dilution water consisted of filtered seawater diluted to a salinity of 29-32 ppt and pH of around 8. The water was vigorously aerated prior to use. Flow-through conditions were calibrated to deliver around 1.2 L of dilution water per oyster per hour.

The test was conducted using 11 L glass aquaria with 6 L dilution water. The photoperiod consisted of 16 h light per day.

All aquaria were examined daily for mortality. At the end of the test, oyster growth was measured to the nearest 0.1 mm. Test water quality was monitored each day. Salinity of the dilution water was measured once daily in the control. Water temperature was measured and recorded hourly. Dissolved oxygen and pH were measured daily. Water samples were collected from the dilution water control and each test solution on days 0, 2 and 4 to monitor actual exposure concentrations.

The 96 h EC50 was calculated using least squares estimation. Statistical differences in growth between the control oysters and exposure concentrations were determined by analysis of variance and Dunnett's test.

Findings:

Mean measured concentrations ranged from 90-108% of nominal over the course of the study, and resulted in mean measured exposure concentrations of 22.8, 40.6, 70.2, 108, 169 and 270 mg ac/L. Water temperature ranged from 21.2-23.3°C and salinity from 29-32 ppt during the study. Dissolved oxygen ranged from 5.7-6.6 mg/L in all exposure vessels and the control throughout the study except for the 22.8 mg/L group on day 2 where it was reduced to 3.5 mg/L due to low flow from a temporary partial occlusion of the water delivery tube. The pH appeared unaffected by 2,4-D addition and remained between 7.8-8.0 throughout the test.

Survival of oysters in all treatments was 100% except the 270 mg/L exposure group where 1 oyster (5%) died. The following shell growth and difference from the controls was measured:

Table A3.50: Shell Growth Changes for the Eastern Oyster

Measured concentration (mg ac/L)	New shell growth (mm)		Percent change
	Treatment mean	Difference from control	
Control	1.2		
22.8	1.1	0.1	-8
40.6	0.9	0.3	-25
70.2	0.8	0.4	-33*
108	0.6	0.6	-50*
169	0.4	0.8	-67*
270	0.2	1.0	-83*

* The mean new shell growth is significantly less than the control mean at alpha = 0.05

After 96 hours exposure, the authors state that no abnormal physical or behavioural effects were noted. However, if an increased respiration rate was responsible for the drop in dissolved oxygen, this should be regarded as a sub-lethal effect from 2,4-D exposure.

Conclusions:

The 96 h EC50 for 2,4-D DMA to oysters was calculated to be 136 mg/L (95% CI 0-388 mg/L. The NOEC was 40.6 mg/L, the highest test concentration with no statistically significant difference in new shell growth as compared to the control group. The 96 h EC50 and NOEC in terms of acid equivalence are around 113 mg ae/L and 34 mg ae/L respectively.

Test Material:	**DMA-4 (49.3% 2,4-D DMA)**
Report:	Heitmuller, 1975
Guidelines:	US EPA Guideline 72-3
GLP:	no

Test system:

Marine toxicity tests were conducted to determine the acute effect of DMA-4 on larvae of the eastern oyster (*Crassostrea virginica*), pink shrimp (*Penaeus duorarum*) and fiddler crabs (*Uca pugilator*) in what appears to have been a static test. The criterion for effect on oyster larvae was failure to develop mormally to the straight hinge stage within 48 hours. The criterion for effect on shrimp was mortality and that for crabs was complete loss of equilibrium. Shrimp and crabs were exposed for 96 hours.

Individual, sexually mature female oysters were induced to spawn in the presence of viable sperm excised from the gonad of a sexually mature male oyster. Density of the larvae was determined by hemacytometer counts and the test containers were each inoculated with 20000-30000 larvae. Concentrations for the definitive 48 h assay were 87, 210, 320, 560 and 750 ppm of product, and all test concentrations along with a control group were duplicated. Test water was filtered natural sea water with salinity adjusted to 23 ppt. Following 48 h exposure, the larvae were collected by sieve, washed and preserved. The number of normal 48-h straight-hinge larvae present in a 200-larvae total count was microscopically determined for each concentration and the control.

For both the shrimp and crab test, water was filtered natural sea water with salinity adjusted to 20 ppt. Temperature was around 20°C for shrimp and 15°C for crabs. Initial pH was around 8.0 for all test concentrations and controls. Each jar contained 5 test animals and all exposure concentrations and controls were duplicated. In the shrimp test, dissolved oxygen was maintained at 40% or greater of saturation.

Shrimp were tested at 0, 420, 650, 750, 870 and 1000 ppm and crabs at 0, 320, 560, 750, 870 and 1000 ppm product. Test substance was pipetted directly into the water in test containers.

Findings:

Dissolved oxygen was not monitored in the oyster larvae test. In the shrimp test it ranged from 41% of saturation in the 420 and 1000 ppm test concentrations to 47% in 750 ppm after 96 h. Dissolved oxygen remained greater than 60% of saturation for all test concentrations and controls in the crab test.

The percentage of abnormally developed oyster larvae was 3, 4, 45, 100, 100 and 100% in the 0, 87, 210, 320, 560 and 750 ppm exposure concentrations respectively.

In the shrimp test, no mortalities were observed in the control or any exposure group up to the maximum 1000 ppm tested at any sampling point. Similarly for crabs, no loss of equilibrium was observed in any control or treatment group throughout the test.

Conclusions:

The 48 h EC50 for oysters is stated in the test report as being between 210 and 320 ppm. No statistical analysis appears to have been conducted. Assuming all toxicity from this product relates to 2,4-D DMA, the EC50 of this chemical would be between 103-158 mg/L (49.3% DMA in the formulation), and on an acid equivalent basis, would be around 85-131 mg ae/L.

The 96 h LC50 for shrimp, and 96 h EC50 for crabs were both >1000 ppm of product (>410.8 mg ae/L).

Apart from data provided to the APVMA for review, several additional tests are described in the US EPA report.

Table A3.51: Additional Freshwater Invertebrate Acute Toxicity Data Reported in US EPA, 2005 Results are in measured concentrations unless otherwise indicated.

Species	Test system	LC50 (mg ac/L)	LC50 (mg ae/L)	MRID
2,4-D DMA Salt				
Daphnia magna	48 h	774.5	642.8	232630
2,4-D Diethanolamine (DEA) Salt				
Daphnia magna	48 h Static	>100	>68	419751-06
2,4-D IPA				
Daphnia magna	48 h Static	583	461	00138869
2,4-D TIPA				
Daphnia magna	48 h Static	630	340.2	

No data were submitted for 2,4-D Sodium salt. However, environmental fate data indicate that the 2,4-D salts and amines rapidly degrade to the acid equivalent.

Table A3.52: Additional Estuarine/Marine Invertebrate Acute Toxicity Data Reported in US EPA, 2005 Results are in measured concentrations unless otherwise indicated.

Species	Test system	LC50 (mg ac/L)	LC50 (mg ae/L)	MRID
2,4-D DMA Salt				
Mysid shrimp (*Americamysis bahia*)	96 h	184	152.7	411583-11[1]
Pink Shrimp (*Panaeus duorarum*)	96 h	>99.6	>82.7	419751-07
Fiddler Crab (*Uca pugilator*)	96 h	1000	830	232630
Grass shrimp (*Palaemonetes pugio*)	96 h	125.9	104.5	232630
2,4-D Diethanolamine (DEA) Salt				
Eastern oyster (*Crassostrea virginica*)	96 h	>112	>76.2	420183-02
Pink Shrimp (*Panaeus duorarum*)	96 h	>99.6	>67.7	419751-07
2,4-D IPA				
Eastern oyster (*Crassostrea virginica*)	96 h	62.8	49.6	414290-03
Pink Shrimp (*Panaeus duorarum*)	96 h	605	478	414290-02
2,4-D TIPA				
Eastern oyster (*Crassostrea virginica*)	96 h	165	89.1	414290-06
Pink Shrimp (*Panaeus duorarum*)	96 h	744	401.8	414290-05

1) DEH has the study with this MRID number (see Alexander *et al*, 1983b). However, there is no reference to Mysid shrimp in the test report.

Aquatic Invertebrates - Chronic

Two chronic *Daphnia* studies were submitted, both using the same active with the following results:

Table A3.53. Summary of Chronic Aquatic Invertebrate Toxicity Results

Test species	Test system	NOEC/MATC (mg ae/L)	Reference
2,4-D DMA *Daphnia magna*	21 d semi-static	46.2/73.1	Mark and Hantink-de Rooy, 1989
Daphnia magna	21 d flow through	22.8/33.6 (m)	Scott Ward, 1991d

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

Test Material:	**2,4-D DMA**
Report:	Mark and Hantink-de Rooy, 1989
Guidelines:	Proposed EC Test Guideline (draft at time of testing)
GLP:	Yes

Test system:

A 21 day study was undertaken on *Daphnia magna* to test the chronic toxicity of 2,4-D DMA using a semi-static system. Animals were 24 h old or less at the commencement of the study. Animals were cultured in natural water supplemented with yeast cells. The water had a pH of 7.9, hardness of 28 mg/L as $CaCO_3$, and total organic carbon of 12.2 mg/L. During the test, the medium was replaced three times per week. Before use, the fresh medium was always aerated to the saturation value for oxygen in water at 20^oC. At renewal, the animals were transferred to freshly prepared test medium and the number of offspring and survival recorded.

The stock solution was prepared by initially dissolving 2.15 g 2,4-D, then subsequently adding 0.52 g DMA slowly to 1 L water. Based on a preliminary test, the exposure concentrations in the definitive test were 21.5, 46.2, 100 and 215 mg ae/L with accompanying DMA concentrations of 5.2, 11.2, 24.2 and 52 mg/L. The control+DMA contained 54 mg DMA/L (the DMA was neutralised with HCl).

At test initiation, 30 animals per test concentration and control were exposed. This was reduced to 20 animals (or as many as survived) on day 7. During the first 5 days, daphnids were kept as a group in a 1 L beaker. On day 5, those with eggs in the brood pouch were transferred to 55 mL vessels containing 44 mL test medium. The remainder of the daphnids stayed in the 1 L beaker. On day 7, the group of individually housed animals was increased to 20 or as many as survived.

No chemical analysis for the determination of the test substance concentrations was performed during the test. Temperature was kept between $19-20^oC$ and 16 hours light per day was maintained. The pH values were measured in the fresh and old medium of the control and test concentrations. The mean reproduction per test group and standard deviation were calculated.

Reproduction per test group and standard deviation were calculated. The difference between two mean numbers of offspring of the test concentrations/control+DMA and

the control without DMA was tested for significance by a one-sided Student t-test for two means.

Findings:

The temperature was kept between 19-20°C. The pH ranged between 7.3-8.8 and the oxygen concentrations varied between 6.4-10.5 mg/L, in most cases. Exceptionally high oxygen concentrations up to 12.7 mg/L were measured and one extremely low value of 0.6 mg/L was observed in the control+DMA on day 5. However, in this case 50% of the test animals had died and the drop of the oxygen concentration was probably caused by the decay of the animals. The following table summarises the reproduction and survival end-points.

Table A3.54: Effects of 2,4-D DMA on *Daphnia magna* in a Chronic Reproduction Study

Concentration [mg ae/L]	Control	DMA control	21.5	46.2	100.0	215.0
Parent survival (%)	95	10	85	90	95	24
Mean young/surviving female	129	53*	140	135	70*	0*
% reproduction[1]		41	109	105	54	0

1) as compared to the control without DMA; * - significantly different (p<0.05).

In terms of survival of parent daphnids, the only significant mortality occurred at the highest concentration tested. However, reproduction was significantly impaired at the 100 mg ae/L concentration. The mortality and reproduction in the control+DMA (54 mg/L DMA) were significantly lower than in the control without DMA.

The time to first brood was not delayed by concentration up to 100 mg/L, however, in the highest concentration of 215 mg ae/L, the first brood was not produced at all. The report notes that in the highest concentration, and in the control+DMA, the digestion of algae was less than in other test concentrations. The higher amount of algae in these groups may result in wide fluctuation of the oxygen concentrations and the pH values in the day and night cycle.

Conclusions:

In terms of parent survival, the LOEC was 215 mg ae/L and the NOEC was 100 mg ae/L (MATC = 157.5 mg ae/L). For reproduction the LOEC was 100 mg ae/L and the NOEC was 46.2 mg ae/L (MATC = 73.1 mg ae/L).

Test Material:	**2,4-D DMA (66.8% pure)**
Report:	Scott Ward, 1991d
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 DMA under flow-through conditions. Daphnids were less than 24 hours old at test initiation and appeared to be in good physical condition. Nominal concentrations tested were 0, 9.1, 18.1, 36.2, 72.5, 145 and 290 mg ac/L and were based on the results of a range finding test. Dilution water had a reported hardness of 65-68 mg/L as CaCO$_3$ and alkalinity of 22-29 mg/L as CaCO$_3$. It was vigorously aerated prior to use. The test was conducted at a temperature range of 18.9-24.5^{o}C.

The test tanks were 11.5 L glass tanks equipped with automatic glass siphons. Within each tank, four replicate test chambers were positioned to receive incoming flow. Each test container maintained 300 mL of test solution. The diluter cycled at an average rate of 2.6 cycles per hour providing approximately 13 volume additions every 24 hours. The test was initiated with the impartial addition of 10 daphnids to each test chamber (40 per treatment). These were positioned in a water bath with lighting regulated to provide 16 hours light per day.

Survival and reproduction was monitored daily and any dead removed. All young produced were counted and discarded. Any abnormalities in the behaviour or physical appearance were noted. Test solutions were not aerated during the test.

Water quality was monitored periodically. Water temperature was continuously monitored. Dissolved oxygen and pH were measured once a week in all test solutions with surviving daphnids. Water hardness and alkalinity of the dilution water and the low and high test concentrations were measured on days 0, 7, 14 and 21. Water samples were collected from the control and each exposure concentration on days 0, 7, 14 and 21 to monitor actual exposure concentrations.

The mortality, reproduction and growth parameters were statistically evaluated by analysis of variance. The 21 d LC50 values and 95% confidence limits were determined by the binomial method.

Findings:

The diluter functioned properly throughout the test and mean measured concentrations ranged from 76-90% of nominal values. The actual mean exposure concentrations were 0, 7.49, 13.9, 27.5, 59.6, 130 and 243 mg ac/L. Test temperature ranged from 18.9-24.5°C. The hardness and alkalinity were 65-68 mg/L and 22-29 mg/L as CaCO$_3$ respectively. Dissolved oxygen concentrations remained at 7.1 mg/L or more in the control but were reduced by the presence of the test substance that appeared to enhance bacterial flora. Dissolved oxygen concentrations in the exposure solutions decreased with increasing concentration, and ranged from 5.7-6.7 mg/L in the 7.49 mg ac/L group, and 2.4-4.8 mg/L in the 130 mg ac/L group. No measurements were taken in the highest test group as all animals were dead. The pH ranged from 6.8-7.4 in all test solutions, and 7.4-7.6 in the control.

Table A3.55: Effects of 2,4-D DMA on *Daphnia magna* in a Chronic Reproduction Study

Concentration [mg/L]	Control	7.49	13.9	27.5	59.6	130	243
Parent Mortality (%)	12	10	32	8	5	90	100
Total young	3007	4153	3390	4414	1487*	0*	-
Average length (mm)	4.20	4.40	4.51	4.39	4.26	3.65	-

1) as compared to the control without DMA; * - significantly different (p<0.05).

Control mortality was relatively high at 12%. At the highest treatment, all daphnids were dead by day 2 and no eggs were produced in this group. However, at the two highest treatments, unhatched eggs were observed extruded with some moults.

Eggs were first observed in the brood chambers of water fleas on test days 5 and 6 in the control and test concentration of 59.6 mg ac/L and less, and on days 6 and 7 in the 130 mg/L group. The average number of young produced per adult based on the initial number of 40 animals were 104, 85, 110, 37 and 0 in the 7.49, 13.9, 27.5, 59.6 and 130 mg ac/L groups respectively. The average number of young produced per adult in the control group was 75.

Mean growth of surviving water fleas (helmet to spine length) was not affected by exposures up to 59.6 mg/L. Growth of the four surviving animals was noticeably less than all other treatments in the 130 mg ac/L group.

Conclusion:

The NOEC was 27.5 mg ac/L (22.8 mg ae/L) based on the lack of significant reproductive impairment at the next highest level. The LOEC was 59.6 mg ac/L (49.5 mg ae/L). The MATC was calculated to be 40.5 mg ac/L (33.6 mg ae/L).

Apart from data provided to the APVMA, the US EPA also reviewed a life-cycle toxicity study to *Daphnia magna* when exposed to the 2,4-D DEA salt with the following result:

Table A3.56: Additional Freshwater Aquatic Invertebrate Life-Cycle Toxicity Data Reported in US EPA, 2005. Results are in measured concentrations unless otherwise indicated.

Species	21 day NOEC/LOEC (mg ae/L)	MATC (mg ae/L)	Endpoints affected	MRID
2,4-D DEA				
Daphnia magna	16.05/25.64	>16.05	Survival and reproduction	420183-03

No other chronic data were reviewed for other amine salts or the sodium salt, and no estuarine or marine species were tested chronically. The need for such studies will be based on the risk assessment from freshwater endpoints.

Algae and Aquatic Plants

Studies were received for a range of species tested with 2,4-D DMA as follows:

Table A.57. Summary of Algae/Aquatic Plant Toxicity Results for 2,4-D Salts

Test species	Test duration	EC50 (mg ae/L)	Reference
2,4-D DMA			
Duckweed (*L. gibba*)	14 days	0.48 (m)	Hughes, 1990g
Green alga (*S. capricornutum*)	120 h	55.2 (m)	Hughes, 1990h
Marine diatom (*S. costatum*)	120 h	129*	Hughes, 1990i
Blue green alga (*A. flos-aquae*)	120 h	127	Hughes, 1990j
Freshwater diatom (*N. pelliculosa*)	120 h	4.38	Hughes, 1990k
Green alga (*S. subspicatus*)	72 h	89	Memmert, 1997c
Green alga (*S. Capricornutum*)	96 h	550	Schoot Uiterkamp, 1989

(m) = measured concentrations; * - DEH Calculated Result

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

Test system:

A study was conducted to assess the effects of 2,4-D DMA on growth inhibition to the duckweed, *Lemna gibba* over 14 days. A stock solution of 2,4-D DMA was prepared

by dissolving in water. Based on the results of a range-finding test, exposure concentrations were set at nominal values of 0, 0.25, 0.5, 1, 2, 4 and 8 mg ac/L. The pH of each treatment was measured. The inoculum of *L. gibba* used to begin the test was taken from 12-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°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:

Measured concentrations were in good agreement with nominal values and were 0, 0.27, 0.53, 0.93, 1.74, 3.64 and 7.60 mg ac/L. Under these static conditions, there was no noticeable decline in concentration over the 14 day test period. The pH values ranged from 8.0-8.1 on day 0 and 8.75-9.3 on day 14.

A summary of frond count findings on day 14 of the test is presented as follows:

Measured Concentration (mg ac/L)	0	0.27	0.53	0.93	1.74	3.64	7.60
Mean frond count (day 14)	503	434	289*	166*	96*	71*	54*
Percent inhibition		14.1	43.7	68.7	82.8	88.1	91.4

* significantly different from the control.

Inhibition commenced with the onset of exposure. For example, after 3 days exposure (the time of first observations), inhibition in the 0.27, 0.53, 0.93, 1.74, 3.64 and 7.60 mg ac/L was 9.5, 16.7, 30.9, 33, 31 and 38% 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 54 at the end), there are no observations as to the health of the plants.

Conclusion:

The 14 day EC25 was determined to be 0.19 mg ac/L (95% CI 0.10-0.37 mg ac/L) and the 14 day EC50 was 0.58 mg ac/L (95% CI 0.37-0.91 mg ac/L). In terms of acid equivalence, the 14 d EC25 and EC50 are 0.16 and 0.48 mg ae/L respectively. The NOEC was 0.27 mg ac/L (0.22 mg ae/L).

Test Material:	**2,4-D DMA**
Report:	Hughes, 1990h
Guidelines:	US EPA Guideline 123-2
GLP:	Yes
Test system:	

A study was conducted to assess the effects of 2,4-D DMA to the green alga, *Selenastrum capricornutum* over 5 days. A stock solution of 2,4-D DMA was prepared by dissolving in water. Based on the results of a range-finding test, exposure concentrations were set at nominal values of 0, 2.5, 5, 10, 20, 40, 80 and 160 mg ac/L. Three replicates of each concentration were performed. The pH of each treatment was measured. The inoculum 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:

Measured concentrations were in good agreement with nominal values and were 0, 2.31, 4.64, 10.3, 19.2, 42.2, 80.3 and 148.4 mg ac/L. Under these static conditions, there was no noticeable decline in concentration over the 5 day test period. The pH values ranged from 7.35-7.6 on day 0 and 7.8-8.4 on day 5.

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

Measured Concentration (mg/L)	0	2.31	4.64	10.3	19.2	42.2	80.3	148.4
Day 5 cells/mL (000s)	4387	3820	3573	3473	3273	2377*	2123*	943*
Percent inhibition	-	12.9	18.5	20.8	25.4	45.8	51.6	78.5

* significantly different from the control.

Conclusion:

The 5 day EC25 was determined to be 25.9 mg ac/L (95% CI 13.4-49.8 mg ac/L) and the 5 day EC50 was 66.5 mg ac/L (95% CI 46.2-95.5 mg ac/L). In terms of acid equivalence, the 5 d EC25 and EC50 are 21.5 and 55.2 mg ae/L respectively. The NOEC was 19.2 mg ac/L (15.9 mg ae/L).

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

Test system:

A study was conducted to assess the effects of 2,4-D DMA 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 DMA was prepared by dissolving in water. Based on the results of a range-finding test, exposure concentrations were set at nominal values of 0, 1.5, 3, 6, 12, 24, 48, 96, 192 and 384 mg ac/L. Three replicates of each concentration were performed. The pH of each treatment was

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measured. The inoculum 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°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 the LSD (least significant difference) test. The level of significance was at 0.05.

Findings:

Measured concentrations ranged from 79-120% of nominal values on day 0 and 97-112% on day 5. Mean measured concentrations were 0, 1.41, 2.81, 6.65, 12.66, 23.38, 49.30, 96.25, 186.7 and 362.6 mg ac/L. Under these static conditions, there was no noticeable decline in concentration over the 5 day test period. The pH values ranged from 8.45-8.55 on day 0 and 7.5-7.85 on day 5.

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

Measured Concentration (mg/L)	0	6.65	12.66	23.38	49.30	96.25	186.7	362.6
Day 5 cells/mL (000s)	421	416	342	514	703	455	36*	18*
Percent inhibition		1.3	18.9	-22.0	-66.8	-7.9	91.5	95.7

* significantly different from the control.

Growth was also much greater than the control at the 1.41 and 2.81 mg ac/L concentrations with 37.3 and 28.2% increases in growth at day 5 compared to the control respectively. From the above results, it is clear that exposure to only the two highest test concentrations had an appreciable inhibitory effect upon the population growth of the diatom when compared to the control.

Conclusion:

The 5 day EC25 was determined to be 15.8 mg ac/L (95% CI 1.8-140.8 mg ac/L) and the 5 day EC50 was 36.6 mg ac/L (95% CI 7.3-183.2 mg ac/L). In terms of acid equivalence, the 5 d EC25 and EC50 are 13.1 and 30.4 mg ae/L respectively. The NOEC was 96.2 mg ac/L (79.9 mg ae/L).

These results, as stated by the author, are obviously contradictory with the EC50 being less than half the NOEC value. The pattern of growth inhibition shown above for day 5 was largely mirrored for the days 3 and 4 results also with significant stimulatory effects at 23.38 and 49.3 mg/L found for all observations. DEH has averaged the inhibitory effects found for days 3, 4 and 5 at the 96.25, 186.7 and 362.6 mg/L groups as these are the concentrations where inhibition was found to occur, and average inhibition was 9.2, 91.0 and 94.4% respectively. (At the next lowest concentration of 49.3 mg ac/L, growth was 60.8% greater than the control).

Plotting Ln(Concentration) against % inhibition for these three highest concentrations, an EC25 and EC50 of around 115 and 155.6 mg ac/L are proposed (equating to around 95.4 and 129 mg ae/L) based on this study.

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

Test system:

A study was conducted to assess the effects of 2,4-D DMA to the filamentous blue green alga, *Anabaena flos-aquae* over 5 days. A stock solution of 2,4-D DMA was prepared by dissolving in water. Based on the results of a range-finding test, exposure concentrations were set at nominal values of 0, 31.25, 62.5, 125, 250, 500 and 1000 mg ac/L. Three replicates of each concentration were performed. The pH of each treatment was measured. The inoculum 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°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:

Measured concentrations ranged from 102-114% of nominal values on day 0 and 104-115% on day 5. Mean measured concentrations were 0, 135.9, 67.86, 130.1, 268.0, 519.8 and 1067 mg ac/L. Under these static conditions, there was no noticeable decline in concentration over the 5 day test period. The pH values ranged from 7.3-7.4 on day 0 and 6.7-7.45 on day 5.

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

Measured Concentration (mg/L)	0	35.90	67.86	130.1	268.0	519.8	1067
Day 5 cells/mL (000s)	343	334	267	154*	88*	124*	75*
Percent inhibition		2.7	22.3	55.2	74.5	64.0	78.1

* significantly different from the control.

Conclusion:

The 5 day EC25 was determined to be 38.5 mg ac/L (95% CI 10.7-139.2 mg ac/L) and the 5 day EC50 was 153.0 mg ac/L (95% CI 67.5-346.9 mg ac/L). In terms of acid equivalence, the 5 d EC25 and EC50 are 31.9 and 127 mg ae/L respectively. The NOEC was 67.86 mg ac/L (56.3 mg ae/L).

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

Report:	Hughes, 1990k
Guidelines:	US EPA Guideline 123-2
GLP:	Yes

Test system:

A study was conducted to assess the effects of 2,4-D DMA to the non-motile freshwater diatom, *Navicula pelliculosa* over 5 days. A stock solution of 2,4-D DMA was prepared by dissolving in water. Based on the results of a range-finding test, exposure concentrations were set at nominal values of 0, 1, 2, 4, 8, 16 and 32 mg ac/L. Three replicates of each concentration were performed. The pH of each treatment was measured. The inoculum 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 $24^{\circ}C$ (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:

Measured concentrations ranged from 90-198% of nominal values on day 0 and 123-147% on day 5. Mean measured concentrations were 0, 1.70, 2.31, 4.27, 10.39, 19.32 and 37.92 mg ac/L. Under these static conditions, there was no noticeable decline in concentration over the 5 day test period. The pH values ranged from 7.3-7.45 on day 0 and 7.55-7.85 on day 5.

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

Measured Concentration (mg/L)	0	1.70	2.31	4.27	10.39	19.32	37.92
Day 5 cells/mL (000s)	853	649	486*	646	133*	198*	33*
Percent inhibition		23.9	43.0	24.2	84.4	76.8	96.1

* significantly different from the control.

Conclusion:

As shown in the 5 day data above, the dose-response pattern is somewhat inconsistent. However, the overall trend is one of increasing inhibition with increasing exposure concentration. The 5 day EC25 was determined to be 2.21 mg ac/L (95% CI 0.73-6.71 mg ac/L) and the 5 day EC50 was 5.28 mg ac/L (95% CI 2.44-11.43 mg ac/L). In terms of acid equivalence, the 5 d EC25 and EC50 are 1.83 and 4.38 mg ae/L respectively. The NOEC was 1.7 mg ac/L (1.41 mg ae/L).

Test Material:	**Herbizid Marks D** (2,4-D 514 g/L solution in its DMA form)
Report:	Memmert, 1997c
Guidelines:	OECD TG 201
GLP:	yes

Test System:

A 72 h toxicity test was conducted to evaluate the influence of the formulation Herbizid Marks D on growth of the freshwater green alga *Scenedesmus subspicatus*.

The test was started by inoculation of a biomass of 10,000 algal cells per mL test medium. The cells were taken from a 3 day old culture. Water temperature was 23-24°C and illumination was continuous. Dilution water had hardness of 24 mg/L as $CaCO_3$. The test design included three replicates per test concentration and six replicates in the control. Test vessels were stirred continuously throughout the study.

The test medium was prepared by dissolving 200 mg of the product in 1000 mL water with intense stirring. From this, the following test concentrations were established: 2.0, 6.3, 20, 63 and 200 mg product/L. Additionally, a control was tested in parallel.

Cell counts ere undertaken after 24, 48 and 72 hours using a Coulter Counter. Three measurements per sample were taken. In addition, a sample was taken from the control and from the two highest test concentrations with reduced algal growth after 72 hours. The shape of these treated cells was microscopically examined and compared with the cells in the control.

At the start and end of the test, the pH was determined in all test concentrations and the control. The water temperature was determined daily in a separate control beaker. The actual exposure concentration of 2,4-D was analysed in one of each duplicate test media samples at 0 and 72 hours. The two lowest test concentrations were not analysed since they were below the determined 72 h NOEC.

Findings:

The analytically determined test substance concentrations in the test medium ranged from 93-97% of the nominal value during the test period. All results are stated in terms of nominal values. The pH values ranged from 7.9-8.0 at the start of the test and had increased to 9.3-10.5 by the end of the test.

The following table shows % inhibition in terms of area under the growth curves (AUC) and growth rate compared to the controls.

Concentration (mg/L)	24 hour % inhibition		48 h % inhibition		72 h % inhibition	
	AUC	Growth rate	AUC	Growth rate	AUC	Growth rate
2.0	1.0	0.3	1.0	0.1	4.1	1.4
6.3	-31.2	-14.1	-18.5	-4.2	-9.0	-0.8
20	-17.9	-6.7	-4.2	0.0	4.4	2.9
63	-25.8	-11.8	-10.1	-1.8	24.7	14.1
200	-27.2	-12.5	17.3	9.7	58.6	38.8

Up to 48 hours, the presence of test compound did not impact on the growth of the algae, in fact, stimulation of growth was apparent at 24 hours and some stimulatory impact still present at 48 hours. However, by 72 hours, statistically significant inhibition of growth was observed at the two highest test concentrations.

Microscopic examination of the shape of the algal cells after 72 hours showed no difference between the algae growing in the test concentrations of nominal 63 and 200 mg/L and those in the control.

Conclusion:

In terms of area under the curve (biomass), the E_bC50 was determined to be 178 mg/L (89 mg ae/L), but 95% confidence limits could not be determined. The growth rate E_rC50 was determined to be 364 mg/L (182 mg ae/L). Again, 95% confidence limits could not be determined. Also, this value should be treated with some caution as the highest inhibition was still less than 50%.

Test Material:	**2,4-D DMA (formulation, 500 g/L 2,4-D)**
Report:	Schoot Uiterkamp, 1989
Guidelines:	OECD TG 201
GLP:	Yes

Test system:

A study was conducted to assess the toxicity of the product U 46 D-Fluid (500 g/L 2,4-D in its DMA salt form) to the freshwater green alga *Selenastrum capricornutum*. The test was conducted over 96 hours. Definitive nominal treatment rates were 0, 1.20, 3.93, 11.9, 39.0, 118, 387 and 1173 mg/L (assumed to be product). Test vessels contained an initial 1040 cells/mL prepared from an algal pre-culture (age of the culture not reported). 1 mL of the appropriate test concentration was added to 99 mL of algal suspension in each test flask. The test was carried out in duplicate with four controls with algae only and a single background control series containing test substance without algae. All flasks were incubated in an orbital shaker at 100 rpm. Continual illumination was used and the temperature was set at 23^oC.

One sample was taken from each flask once a day for four consecutive days and the number of algal cells per mL determined. After 71.5 and 93.5 h of the growth inhibition test, the pH in the different cultures was measured. The morphology of the algae was examined microscopically at the end of the test.

The EC50 was calculated by means of parametric analysis. The NOEC was estimated by comparison of the growth curves of the treated algal suspensions with those of the controls.

Findings:

The range-finding test revealed that inhibiting effects could be expected at concentrations >120 mg/L. It is reported that the pH value of the medium increased only slightly during the first three days of the test. No pH measurements are provided in the test report except in the characterisation of the initial algal medium where it is reported as being around 8.

Nominal Concentration (mg/L)	0	1.20	3.93	11.9	39.0	118	387	1173
Cells (10^4)/mL	345.8[1]	340.7	346.8	355.7	308.6	310.9	208.0	5.6
% Inhibition	-	1.47	-0.29	-2.86	10.76	10.1	39.85	98.38

1) mean value of both control groups.

Microscopic examination of the cells at the start and end of the incubation period revealed no abnormal cells except for the highest concentration at the end of the test where mostly abnormal cells were found.

Conclusion:

Of several parametric models available, the model that assumes an effect on the growth rate appeared to fit the data. The EC50 with respect to growth rate was found to be 1100 mg/L with 95% confidence limits of 920-1400 mg/L. By comparison of the growth curves, the NOEC of product was estimated to be 118 mg/L. In terms of 2,4-D Acid, the EC50 and NOEC are around 550 and 59 mg ae/L respectively.

In addition to test data provided to the APVMA, several studies have been reviewed by the US EPA. 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. No Tier I testing was performed for 2,4-D salts, rather, registrants moved straight onto Tier II testing.

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. No comments can be made on these results as the test reports have not been reviewed by DEH.

Table A3.58: Additional Non-target Aquatic Plant Toxicity (Tier II) Data Reported in US EPA, 2005. Measured Concentrations.

Species	EC50/NOEC (mg ac/L)	EC50/NOEC (mg ae/L)	MRID
2,4-D DEA			
Duckweed *Lemna gibba*	0.44/0.07	0.30/0.05	427122-04
Green algae *Selenastrum capricornutum*	11/0.50	7.48/0.34	427122-05
Marine diatom *Skeletonema costatum*	>95/95	>64.6/64.6	427122-01
Freshwater diatom *Navicula pelliculosa*	>97/97	>66/66	427122-02
Blue-green algae *Anabaena flos-aquae*	>96/96	>65.3/65.3	427122-03
2,4-D IPA			
Green algae *Selenastrum capricornutum*	43.4/13.9	34.29/10.98	417321-02
2,4-D TIPA			
Duckweed *Lemna gibba*	2.37/2.38	1.28/1.28	434886-02
Green algae *Selenastrum capricornutum*	75.7/55.4	40.88/29.92	417321/01
Marine diatom *Skeletonema costatum*	79.7/50.4	38.29/24.21	434886-03
Freshwater diatom *Navicula pelliculosa*	94.4/5.35	50.98/2.89	434886-01
Blue-green algae *Anabaena flos-aquae*	133/47.9	71.82/25.87	434886-04

Aquatic plant toxicity data are lacking for the 2,4-D Sodium Salt.

Conclusions for Aquatic Toxicity

Several acute fish toxicity results were reviewed for 2,4-D DMA salt and one for 2,4-D sodium salt. Results were in good agreement that both these salts were practically

non-toxic to fish (96 h LD50s >100 mg ae/L) with the exception of a non-standard test using 2,4-D DMA salt on the non-standard species, Mozambique tilapia with a 96 h LC50 of 35.6 mg ae/L (slightly toxic). A total of eighteen other non-reviewed results for the 2,4-D DMA, DEA, IPA, TIPA and Sodium salts all added to the weight of evidence that 2,4-D in its salt forms will not be toxic to fish with all definitive results showing 96 h LC50s >80 mg ae/L. A single acute amphibian test on leopard frog tadpoles for 2,4-D DMA salt again resulted in a conclusion of low toxicity with the 96 h LC50 of 188 mg ae/L. Two chronic studies reviewed for 2,4-D DMA resulted in a 28 d NOEC of 100 mg ae/L to rainbow trout and a 31 d MATC of 18.3 mg ae/L to fathead minnow while results of one non-reviewed test showed a MATC of 36.3 mg ae/L. Both indicate very slight toxicity.

Several acute aquatic invertebrate toxicity results were reviewed for 2,4-D DMA salt and all results tended to support a conclusion of this compound being practically non-toxic to aquatic invertebrates with defined LC50s >100 mg ae/ha. One older study resulted in an EC50 somewhere between 85-131 mg ae/L. In addition, fourteen non-reviewed tests provided results generally supportive of limited toxicity. The most sensitive species tested appeared to be the eastern oyster with a 96 h LC50 of 49.6 mg ae/L with 2,4-D IPA and 89.1 mg ae/L for 2,4-D TIPA (both slightly toxic). Generally, however, defined LC50s were >100 mg ae/L for non-reviewed studies for 2,4-D DMA, DEA, IPA and TIPA salts. Two chronic test results, both to *Daphnia magna*, were reviewed resulting in MATC values of 73.1 and 33.6 mg ae/L. This very slight toxicity was supported with a non-reviewed test result of an MATC >16.05 mg ae/L for 2,4-D DEA salt to *Daphnia magna*.

Algae and aquatic plants were the most sensitive aquatic organisms to 2,4-D salts. Seven standard tests for different algae and the duckweed *Lemna gibba* were available for 2,4-D DMA along with supporting results for the 2,4-D DEA, IPA and TIPA salts. Generally, these salts were slightly to practically non-toxic to the simpler freshwater and marine algae. The most sensitive algae species appeared to be the freshwater diatom *N. pelliculosa* with an EC50 of 4.38 mg ae/L from 2,4-D DMA salt, and the green alga *S. capricornutum* with an unreviewed EC 50 of 7.48 mg ae/L to the 2,4-D DEA salt (both moderately toxic). The vascular aquatic plant, duckweed (*L. gibba*) was more sensitive with 2,4-D DMA and DEA salts being highly toxic to this plant with EC50s of 0.48 and 0.30 mg ae/L respectively. The only other salt for which a result is available to duckweed was 2,4-D TIPA with an EC50 of 1.3 mg ae/L.

Terrestrial Toxicity

Non-Target Invertebrates
Bees

Four studies (2 being non-standard) were provided with the following results:

Table A3.59. Summary of Toxicity to Bees for 2,4-D in Various Forms

Test species	Test duration	LD50 (µg ae/bee)	Reference
2,4-D DMA			
Honey bee (*A. mellifera*)	72 h oral	78	Hoxter *et al*, 1997b
Honey bee (*A. mellifera*)	48 h contact	>83.3	Palmer and Krueger, 1997e
Worker bee (*A. indica*)	24 h contact	0.136% v/v*	Jeyalakshmi, 2002c
2,4-D Sodium salt			
Worker bee (*A. indica*)	24 h contact	>3% w/v*	Jeyalakshmi, 2002d

* Non-standard test

Test Material:	**2,4-D DMA (67.3% Purity)**
Report:	Hoxter *et al*, 1997b
Guidelines:	EPPO Guideline No. 170
GLP:	Yes

Test system:

The study was undertaken to evaluate the acute oral toxicity of 2,4-D DMA salt administered to the honey bee (*Apis mellifera*) in the diet. Honey bees were exposed to a geometric series of test concentrations or 0, 6.25, 12.5, 25.0, 50.0 and 100 µg ac/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°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 0.75, 4, 24, 48 and 72 hours.

The LD50 values were calculated by the binomial method in the treatment group and probit analysis in the positive control group. After mortality in the treatment groups was corrected for negative control mortality, LD50 values could not be calculated with standard statistical techniques, so the LD50 values for the adjusted mortality data were 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 63-88%. Two bees in the negative control group were observed as immobile at the second observation on day 0. Otherwise, all surviving bees in the control group remained normal in appearance and behaviour throughout the test. Mortality in the negative control group was 17% by the end of the study. In the

positive control, 7, 45 and 93% 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 7, 7, 7, 7 and 55% respectively. When mortality in the highest group was adjusted for control mortality, it was around 46% at 72 hours.

Sub-lethal effects were noted in the lowest treatment group with 1 bee being lethargic at 72 hours. In the 12.5 μg/bee treatment level, 1 bee was lethargic and 1 bee immobile after 72 hours. However, these effects are unlikely to be treatment related as no other sub-lethal effects were found in the higher treatment groups.

Conclusion:

The 72 h acute oral LD50 value for the honey bee exposed to 2,4-D DMA salt was determined to be approximately 94 μg ac/bee with a lower 95% CI of 50 μg ac/bee. The NOEC was 50 μg ac/bee. In terms of acid equivalence, the LD50 and NOEC are 78 and 41.5 μg ae/bee respectively.

Test Material:	**2,4-D DMA (67.3% Purity)**
Report:	Palmer and Krueger, 1997e
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 DMA salt administered to the honey bee (*Apis mellifera*). Honey bees were exposed to a geometric series of test concentrations of 0, 6.25, 12.5, 25.0, 50.0 and 100 μg ac/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°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 0.75, 3.25, 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 27.3-27.5°C while relative humidity range from 55-81%. At test termination there was 2% mortality among bees in the negative control group, while there were no mortalities 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.

The only mortality observed in the treatment groups was 3% in the 100 µg/bee group. With the exception of one bee in this group that was immobile on day 0, all other surviving bees in the five treatment groups were normal in appearance and behaviour throughout the test period.

Conclusion:

The 48 h acute contact LD50 for honey bees exposed to 2,4-D DMA salt was determined to be >100 µg ac/bee (>83.3 µg e/bee). The NOEC was 100 µg ac/bee.

Test Material:	**2,4-D DMA SL Salt**
Report:	Jeyalakshmi, 2002c
Guidelines:	Gaitonde Committee Guideline No. 6.6.0
GLP:	No (Quality Assurance Statement provided).

Test system:

Toxicity of 2,4-D DMA salt (59.1% w/w 2,4-D) to worker bees (*Apis indica*), 5-15 days old, was tested using a dry film method. Twelve treatments were performed with 2,4-D acid at 0.08, 0.10, 0.20, 0.40 and 0.60% w/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 replicte.

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 acid treatment groups, mortality in the 0.08, 0.10, 0.20, 0.40 and 0.60% w/v treatment groups after 24 hours (mean of three replicates) was 3.3, 33.3, 53.3, 63.3 and 83.3% respectively.

Conclusion:

The LC50 for 2,4-D DMA SL salt was calculated to be 0.230% v/v with confidence limits (assumed to be 95%) of 0.204-0.256% v/v. In terms of acid equivalence, the LC50 would be 0.136% 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.

Test Material:	**2,4-D Sodium salt**
Report:	Jeyalakshmi, 2002d
Guidelines:	Gaitonde Committee Guideline No. 6.6.0
GLP:	No (Quality Assurance Statement provided).

Test system:

Toxicity of 2,4-D sodium salt (80% WP formulation) to worker bees (*Apis indica*), 5-15 days old, was tested using a dry film method. Thirteen treatments were performed with the formulation at 0.5, 1, 1.5, 2.0, 2.5 and 3.0% w/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 replicte.

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^oC) 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 acid treatment groups, mortality in the 0.5, 1, 1.5, 2.0, 2.5 and 3.0% w/v treatment groups after 24 hours (mean of three replicates) was 16.7, 26.7, 30.0, 30.0, 33.3 and 36.7% respectively.

Conclusion:

Because less than 50% mortality was found at the highest treatment level, an LC50 could not be calculated. While almost 40% mortality was found at 3% w/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

Several studies to terrestrial arthropods were provided with the following results:

Table A3.60. Summary of Toxicity to Terrestrial Arthropods for 2,4-D in Various Forms

Test species	Test system	IOBC Classification	Reference
2,4-D DMA			
Predatory mite (*T. pyri*)	14 d laboratory	Harmless up to 1800 g ae/ha	Kuhner, 1998c
Predatory mite (*T. pyri*)	14 d laboratory	Harmless up to 1000 g ae/ha	Goβmann, 1997a
Parasitic wasp (*A. rhopalosiphi*)	14 d laboratory	Harmless up to 1800 g ae/ha	Kuhner, 1998d
Spiders (*Pardosa* sp.) (7 species)	14 d laboratory	Harmless up to 1056 g ae/ha	Schmitzer and Breitwieser, 1997
Rove beetle (*A. bilineata*)	28 d laboratory	Harmless up to 1000 g ae/ha	Goβmann, 1997b

Test Material:	**2,4-D DMA (600 g/L ae formulation)**
Report:	Kuhner, 1998c
Guidelines:	LOUIS/UFER, 1995
GLP:	Yes

Test system:

The study was undertaken to determine the effect of the formulation Desormone liquid (600 g ac/L 2,4-D as the DMA salt) on the predatory mite *Typhlodromus pyri* in the laboratory. The application rate was based on the maximum spraying volume (3 kg/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 spraying-cabin. 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.

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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, 89% of mites were alive in the direct spray group with 86% alive in the 5% spray drift group. This compared to 94% in the control group and 6% in the positive control. The corrected mortality therefore for the 3 kg/ha and 0.3 kg/ha groups were 5.3 and 8.5% respectively. Both results are considered to be within the natural variability of the test system.

Regarding fecundity, during the 7 day egg laying period, the number of offspring per female in the control was 8.0 compared to 7.9 and 7.8 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 6.2% and 10.3% respectively.

Conclusion:

Based on these results, the formulation should be classified as harmless to *T. pyri* according to IOBC categories when applied at 3 kg/ha product. In terms of active, this relates to an application rate of 1800 g ae/ha.

Test Material:	**Herbizid Marks D** (2,4-D DMA, 514 g ae/L solution)
Report:	Goβmann, 1997a
Guidelines:	IOBC/WPRS guidelines, and LOUIS/UFER, 1995.
GLP:	Yes

Test system:

The study was undertaken to determine the effect of the formulation Herbizid Marks D (500 g ac/L 2,4-D as the DMA salt) on the predatory mite *Typhlodromus pyri* in the laboratory. A single application rate equivalent to 2 L formulated product in 200 L/ha water was used (equivalent to around 1000 g 2,4-D/ha). Dimethoate was applied as a toxic standard to confirm efficacy of the test system. Each exposure group included 5 replicates containing 20 mites each. Application was made using a laboratory sprayer. After application the test units were allowed to dry for a period of around 2 hours prior to assembly of the test containers and introduction of the test organisms.

The study aimed to assess mortality of protonymphs (around 3 days 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 on days 1, 3 and 7. The number of males and females surviving in each replicate was determined on day 7. In addition, live and dead individuals were counted at days 9, 11 and 14.

To consider reproductive effects, one week after test initiation, the surviving individuals were sexed. The number of eggs layed in each container together with the juvenile stages found and the number of female and male predators were counted on days 7, 9, 11 and 14. Reproduction of the mites was determined by calculating the

number of eggs and live and dead juvenile stages per surviving female on the test units.

Test conditions of temperature, humidity and photoperiod are not provided in the test report.

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.

After 7 days, mortality in the negative control was 12%. The test substance mortality was 35% taking mortality as a whole across the 5 replicates. However, the majority of this mortality (20%) came from one replicate where all the mites had died. Average mortality in the other 4 replicates was 18.8% and ranged from 10-30% in any one replicate (compared to a range of 5-20% in any one replicate in the control groups). Given the consistency amongst other replicates, it is difficult to attribute the high mortality in one replicate as being a result of the test substance. Rather, it may have been that this replicate was compromised in some manner, and the results will be disregarded in analysing the overall findings of the study. In the toxic control, mortality after the first week was 98%. Given the control mortality, the corrected mortality for the treated groups (ignoring the outlier) was 7.67%.

The mean egg production per female of the mites exposed to residues of the formulation during the second week amounted on total to 5.3 (ignoring the outlier replicate). This compared to 6.8 eggs per female in the tap water control and was not considered statistically different based on Dunnett's test at a significance level of 0.05.

Conclusion:

Ignoring the outlier replicate in the treated group, the overall reduction in beneficial capacity was calculated to be 27.98%. According to IOBC classification, the formulation Herbizid Marks D is classified as harmless (bordering on slightly harmful) at an application rate of 2000 g/ha (1000 g ae/ha).

Test Material:	**2,4-D DMA (600 g/L 2,4-D formulation)**
Report:	Kuhner, 1998d
Guidelines:	Polgar, 1988; Mead-Briggs, 1992
GLP:	Yes

Test system:

The study was undertaken to determine the effect of the formulation Desormone liquid (600 g ae/L 2,4-D as the DMA salt) on the aphid parasitoid *Aphidius rhopalosiphi* in the laboratory. The application rate was based on the maximum spraying volume (3 kg/ha product) 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, 5, 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°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, 2 adults were dead and 1 was moribund (7.5%) in the high treatment and all were alive in the low treatment. 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 both the high and low treatment groups. They produced 63 and 87 mummies respectively, resulting in 4.2 and 5.8 mummies per female respectively. The reduction in reproduction rate based on these results was 6.1% and –29.6% in the high and low treatment groups respectively.

The combination of mortality and reproduction rate resulted in an overall reduction in beneficial capacity of 13.2 and –29.6% in the high and low treatment groups respectively.

Conclusion:

Based on these results, the formulation should be classified as harmless to *A. rhopalosiphi* according to IOBC categories when applied at 3 kg/ha. In terms of active, this relates to an application rate of 1800 g ae/ha.

Test Material:	**Herbizid Marks D** (2,4-D DMA, 514 g ae/L solution)
Report:	Schmitzer and Breitwieser, 1997
Guidelines:	BBA Draft Guideline, 1994
GLP:	Yes

Test system:

The study was undertaken to estimate the effect of the formulation, Herbizid Marks D (500 g/L 2,4-D in the DMA salt form) on the mortality, behaviour and predatory performance of the spiders, as compared to water treated controls and an endosulfan toxic standard. The study was performed with 7 spider species, *Pardosa palustris,*

Pardosa prativaga, Pardosa pulata, Pardosa agricola, Pardosa agrestris and *Pardosa hortensis* collected from uncultivated land. The test was performed with adult individuals that were not in the reproductive phase. They were acclimatised for 12 days using a 16:8 hour light:dark photoperiod at room temperature. Up to 5 spiders were kept in cages filled with moistened clay pellets.

Test units consisted of white plastic trays containing a layer of dry quartz sand. Sand was moistened to around 70% maximum water capacity and the spiders were added 3 days before application. Test conditions were temperature 19-21°C, 45-92% relative humidity and 16:8 h light:dark photoperiod.

The test substance was emulsified in tap water at a concentration of 5.86 g/L. A laboratory sprayer applied the spray onto the sand at an amount of around 4 mg/cm^2 equivalent to an application rate of around 1056 g 2,4-D/ha.

20 trays per treatment were used consisting of 10 males and 10 females in separate trays. During the 3 days prior to application, the spiders were not fed. Following application, they were fed with 5 *Drosophila hydei* per individual. They were fed routinely throughout the experiment which ran for 14 days.

At 2,4 and 6 hours after application spiders were observed. From 6 h onwards, spiders showing sub-lethal symptoms were placed at a corner of the tray for observation of possible regeneration. Examination was then daily until day four and then observations were made on days 7, 9, 11 and 14. Because not more than 2 spiders died between days 7-14, the experiment was finished after 2 weeks. At days 1, 2, 3, 4, 7, 9, 11 and 14, food consumption was recorded. Average food consumption in the second week was not reduced by 50% or more as compared to the water control spiders.

The test was considered valid as mortality of the control spiders did not exceed 10% and there was >50% mortality in the toxic standard spiders.

Findings:

Analytical dose verification of the test substance in the spray solution showed recovery of 103% (2.64 g 2,4-D/L).

One spider died in the formulation treated group and the control group over the course of the study (5% mortality for each). No behavioural abnormalities occurred throughout the course of the experiment in both treatment groups. In the toxic standard group, 100% mortality was found after 7 days.

Food consumption was similar among the water control and formulation exposed spiders. A mean of 16 flies/spider were consumed in the treated group (18/female and 15/male) over the two weeks. This compared to 14 flies/spider in the control group (17/female and 11/male).

Conclusion:

2,4-D when applied as the formulation Herbizid Marks D had no adverse effect on mortality, behaviour or food consumption of spiders (*Pardosa sp.*) when applied up to 1056 g/ha.

Test Material:	**Herbizid Marks D** (2,4-D DMA, 514 g ae/L solution)
Report:	Goβmann, 1997b
Guidelines:	IOBC/WPRS 1992.
GLP:	Yes

Test system:

The study was undertaken to determine the effect of the formulation Herbizid Marks D on the reproduction of the rove beetle, *Aleochara bilineata* in a laboratory experiment. Beetles were around 3 days old at the start of the test. 10 pairs of beetles were exposed.

The test units consisted of glass beakers approximately half filled with a layer of sand moistened to 10% v/v with tap water. The beakers' walls were covered with filter paper prior to application and removed afterwards to ensure no higher concentration was applied. The cages were exposed at room temperature (around 20°C) with a photoperiod of 16 hours. The beetles were fed 5 times a week and the moisture of the sand was checked weekly.

Application was equivalent to 2 L product/ha (1000 g ae/ha). In addition, a negative control (water) and positive control (dimethoate) were applied. Before the cages were sprayed, the beetles were put in test cages into a hole dug in the sand, then covered. The test was performed in three replicates (10 pairs of beetles each) for all groups. It lasted until hatching of the new F1-generation of beetles had finished (about 9 weeks). At day 8, 15 and 22 around 500 onion fly pupae per cage were carefully introduced into the sand. At the same time, moisture content of the sand was checked and amended if necessary. All observed behavioural abnormalities of the beetles were recorded.

After four weeks the fly pupae were washed out of the sand and transferred to separate containers. Hatched *A. bilineata* of the new generation were counted at least 3 times a week.

The study was considered valid because the reproduction rate of the water control was at least 30 beetles per female and in the toxic standard was a reduction of reproduction of 48%.

Findings:

Analytical dose verification of the test substance was conducted and showed recovery of 101%

No behavioural abnormalities or intoxication symptoms on the beetles exposed to the treatment were observed. Hatching of the F1 generation started about 5 weeks after the start and the last beetles hatched after around 9 weeks. A mean of 352 young hatched in the group treated with the test formula compared to a mean of 356 young in the control and 186 in the positive control.

Conclusion:

The reduction in reproductive capacity of 1.3% of rove beetles exposed to Herbizid Marks D compared to control beetles is not considered statistically different. The formulation can be considered as harmless to rove beetles based on IOBC classification when treated up to 2000 g product/ha (1000 g ae/ha).

Earthworms

Test Material:	**2,4-D DMA (500 g/L ae formulation)**
Report:	Adema and Roza, 1989
Guidelines:	OECD Guideline 207
GLP:	Yes

Test system:

The study was undertaken to determine the acute toxicity of the formulation U 46 D-Fluid (2,4-D at 500 g/L as the DMA salt) towards the earthworm *Eisenia fetida*. The test was conducted over 14 days at concentrations of 0, 10, 32, 100, 320 and 1000 mg product/kg dry soil. Artificial soil consisting of peat, clay and fine sand in the ratio of 1:2:7 was used. These components were mixed with distilled water and calcium carbonate. The pH and moisture content as determined before introduction of the worms was 5.7 and 54.8% (based on dry weight) respectively.

The test was performed in quadruplicate in 1 L glass containers, each containing about 750 g wet weight of the artificial soil and 10 worms. Mortality was determined after 7 and 14 days. At the same time, appearance and behaviour was visually assessed. After the mortality determination on the 7th day, the worms and medium were replaced in their test containers. After the 14th day, the weight of the worms was determined. At the end of the test, the moisture content was found to be 56.1% based on dry weight. The test was carried out at around 21°C and under continuous illumination.

The LC50 values and their confidence limits were calculated by means of a parametric model.

Findings:

At test concentrations of 10, 32 and 100 mg/kg product, the test worms appeared and behaved the same as the control worms. At 320 mg/kg product, the surviving worms appeared poorer than those of the control worms while their behaviour was poorer than the control worms (sluggish or irritated). Complete mortality was found at the highest test rate of 1000 mg/kg product.

The following summarises mortality and weigh data after 14 days.

Exposure (mg/kg dry wt)	0	10	32	100	320	1000
Mortality (%)	0	0	0	0	15	100
Weight per batch (g)	0.55	0.56	0.521	0.530	0.41	-

Average worm weight in the 320 g/kg group was around 24% less than control worms.

Conclusion:

The 14 d LC50 was calculated to be 700 mg/kg soil of product, equating to 350 mg ae/kg soil. The 14 d NOEC was determined by the authors to be 200 mg/kg soil or product (100 mg ae/kg soil).

Test Material:	**Herbizid Marks D** (2,4-D DMA, 514 g ae/L solution)
Report:	Goβmann, 1997c
Guidelines:	OECD Guideline 207; ISO Guideline 11268 part 1.
GLP:	Yes

Test system:

The study was undertaken to determine the acute toxicity of the formulation Herbizid Marks D (2,4-D at 500 g/L as the DMA salt) towards the earthworm *Eisenia fetida*. Mortality and changes in earthworm body weight were used as toxic endpoints. Sub-lethal effects were also monitored.

The test was conducted over 14 days at concentrations of 0, 1.0, 10, 100 and 1000 mg product/kg dry soil. Artificial soil consisting of peat, clay and fine sand in the ratio of 1:2:7 was used. These components were mixed with distilled water and calcium carbonate. The moisture content was adjusted to 40-60% water capacity and the pH was 6.0 at the start of the study. After the end of the study, these parameters were re-determined.

The test was performed in quadruplicate in 1 L glass containers containing artificial soil and 10 worms each. Mortality was determined after 7 and 14 days. At the same time, appearance and behaviour was visually assessed. After the 14th day, the weight of the worms was determined. The test was carried out at a temperature of 17-21°C and under continuous illumination.

The LC50 values and their confidence limits were calculated by probit analysis or moving mean interpolation.

Findings:

At the beginning of the study, the pH of the soil was 6.26-6.31 with a range of 5.78-5.88 at the end. The water content was 31.7-32.8% (dry weight) at the beginning of the test and 30.4-31.2% at the end.

No toxic effects from acute exposure of the formulation to earthworms was observed. Only one worm, from the 0.1 mg/kg treatment level, died during the experiment and this was not considered treatment related. In all treatment groups, the body weights of the surviving worms decreased slightly due to the starvation of the animals within the test period. No behavioural abnormalities were observed.

Conclusion:

The LC50 of the formulation Herbizid Marks D to earthworms in artificial soil was determined to be >1000 mg/kg (correlating to a nominal 500 mg ae/kg soil). The NOEC was 1000 mg/kg (500 mg ae/kg soil).

Soil Micro-organisms

Test Material:	**2,4-D DMA (formulation, 500 g/L 2,4-D)**
Report:	Zohner, 1989a
Guidelines:	BBA Guidelines, Part IV, 1-1
GLP:	Yes

Test system:

A study was conducted to assess the effects of direct introduction of 2,4-D DMA on soil respiration for a period of 4 weeks. The formulation used was U 46 D-Fluid, containing 500 g/L 2,4-D in the form of 2,4-D DMA. Two representative agricultural soils were used. These German soils were classified as a sandy loam (Eferding soil) and silt loam (Auboden soil) with the following soil characteristics:

Series	% Sand	% Silt	% Clay	pH	%OC[1]	Biomass mg CO_2/100 g
Eferding	66.7	25.2	8.1	7.6	0.9	2.34
Auboden	17.6	68.2	14.2	7.0	1.6	4.2

1) Soil characteristic sheets state this as % Hu.

To prepare the soils, an excess amount of soil was air dried, sieved (2 mm), and adjusted to 40% maximum water capacity and pre-incubated for 2 weeks before use for restoration and equilibration of microbial activity.

Application rates were based on an assumed highest application rate and 10 times this. The highest rate quoted by the study authors for this formulation is 2 L/ha. Assuming a soil depth of 5 cm and an arbitrary density of 1.5 g/cm³, these rates resulted in tested concentrations of 1.33 and 13.30 mg ae/kg soil. The test substance was prepared by dissolution in water and was added to 600 g soil (at 40% water holding capacity). The soil samples were homogenised with an electric stirrer. From the 600 g soil, 170 g subsamples were filled in 250 mL aerated glass flasks in three replicates for each group.

Respiration test: Soil respiration was measured by determination of the CO_2 released from soil samples continuously purged with water saturated CO_2 free air. The temperature was kept at around 20°C and monitored continuously. CO_2 was trapped in a KOH trapping solution with levels determined by titration after 2, 7, 14, 21 and 28 days. Three CO_2 traps were provided for blank titration samples.

Overall, six groups of the study were performed, namely: soil without lucerne (control); soil with 0.5% lucerne (control); soil treated with 1 X test substance; soil treated with 10 X test substance; soil with 0.5% lucerne and treated with 1 X test substance; and soil with 0.5% lucerne and treated with 10 X test substance.

Findings:

The microbial biomass of soil 1 and 2 was 2.3 and 4.2 mg CO_2/100 g/h respectively at time of field sampling and 2.2 and 3.7 mg CO_2/100 g/h respectively at the beginning of incubation.

Table A3.61: Soil Respiration Findings

Period (Days)	Eferding soil			Auboden soil		
	Control	1.33 mg ae/kg	10.3 mg ae/kg	Control	1.33 mg ae/kg	10.3 mg ae/kg
Soils not amended with lucerne (measured in mg CO_2/100 g dry wt. soil/period)						
0-2	8.7	8.6	9.2	6.8	9.1	8.8
2-7	14.1	12.5	12.6	11.3	17.7	15.1
7-14	13.8	12.8	13.2	11.56	12.3	12.5
14-21	12.2	12.0	12.0	9.7	12.0	10.5
21-28	13.4	10.6	10.7	12.2	7.6	11.9
Soils amended with lucerne (measured in mg CO_2/100 g dry wt. soil/period)						
0-2	127.9	147.6	131.8	93.6	108.3	120.0
2-7	153.3	141.2	152.1	163.4	147.5	138.9
7-14	73.3	70.9	75.5	66.2	68.4	64.2
14-21	42.5	37.1	42.8	35.7	35.0	35.0
21-28	26.0	31.5	30.0	26.2	23.1	22.6

Soils amended with lucerne showed significantly greater respiration rates than unamended soils. On the unamended soils treated with 1 X test substance, a respiration of 79.4% (soil 1) and 62.2% (soil 2) were found compared to the control after 4 weeks. The respective figures of the 10 X treated soils were 79.8% and 98.2%.

In the amended soils treated with 1 X test substance, a respiration rate of 121.2% (soil 1) and 88.3% (soil 2) was found compared to the control after 4 weeks. The respective figures of the 10 X treated soils were 115.4% and 86.2%.

Conclusions:

The authors discuss the results based on a German assessment scheme where deviations of microbial activity from 0-15% compared to the control are neglected, and deviations from 15-50% after 4 weeks are tolerated. OECD TG 217 states that where the difference in respiration rates between the lower treatment and control is equal to or less than 25% at any sampling time after day 28, the product can be evaluated as having no long-term influence on carbon transformation in soils.

In this study, while a deviation of −37.8% was found at 1X test substance in the non-amended soil 2 at 4 weeks, this did not appear to be treatment related as the 10 X rate on the same soil at 4 weeks was only −1.8% (an acceptable deviation). The outcomes of this study indicate that 2,4-D should have no significant impact on soil respiration when applied at a rate up to 13.3 mg ae/kg soil.

Test Material:	**2,4-D DMA (formulation, 500 g/L 2,4-D)**
Report:	Zohner, 1989b
Guidelines:	BBA Guidelines, Part IV, 1-1
GLP:	Yes

Test system:

A study was conducted to assess the effects of direct introduction of 2,4-D DMA on the ammonification and nitrification of soil for a period of 4 weeks. The formulation used was U 46 D-Fluid, containing 500 g/L 2,4-D in the form of 2,4-D DMA. The soils used, test system and application rates were as described above for Zohner 1989a. However, the following differences are noted:

The test substance was prepared by dissolution in water and was added to 2000 g soil (at 40% water holding capacity). Lucerne meal was amended to 0.5% and the soil samples were homogenised. No testing was performed on non-amended soils. Soils were analysed in three replicates.

To extract the soils, 75 g of moist soil was extracted by shaking with 300 mL 0.025 N calcium chloride solution. Determination of ammonium-N and nitrate-N were performed. Nitrite-N was not analysed because no significant difference was found between the sum of ammonium-N and nitrate-N in the treated samples and the control sample.

Findings:

The microbial biomass of soil 1 and 2 was 2.3 and 4.2 mg CO_2/100 g/h respectively at the initiation of incubation. NO_3-N was the predominating N-form in the soil extracts. Concentrations of NH_4-N were negligible at all sampling dates. Therefore, the N-transformation was described by the nitrate content solely in the respective soil samples and are summarised in the following table:

Table A3.62: Soil Ammonification Findings – Nitrate Concentrations in Soil.

Period (Days)	Eferding soil			Auboden soil		
	Control	1.33 mg ae/kg	10.3 mg ae/kg	Control	1.33 mg ae/kg	10.3 mg ae/kg
0	1.7	1.7	1.7	2.0	2.0	2.0
7	2.4	2.3	2.5	3.2	3.0	3.3
14	3.9	3.9	4.3	4.3	4.3	4.3
21	5.3	5.1	5.3	5.2	5.3	5.4
28	5.7	5.6	5.6	5.6	5.4	5.6

The N-mineralisation of the 1X treated soils was 98.6-98.1% compared to the controls after 4 weeks. The respective figures of the 10X treated soils were 98.8-100.8%.

Conclusions:

The authors discuss the results based on a German assessment scheme where deviations of microbial activity from 0-15% compared to the control are neglected, and deviations from 15-50% after 4 weeks are tolerated. OECD TG 216 states that where the difference in respiration rates between the lower treatment and control is equal to or less than 25% at any sampling time after day 28, the product can be evaluated as having no long-term influence on carbon transformation in soils.

The small deviations from the control in terms of nitrogen transformation from this study indicate that 2,4-D should have no significant impact on the ammonification and nitrification of soil when applied at a rate up to 13.3 mg ae/kg soil.

Non-Target Terrestrial Vegetation

Only one study was provided for seedling emergence with exposure to the 2,4-D DMA salt. No vegetative vigour studies were provided, although this is the growth stage where more sensitivity may be expected.

Test Material:	**2,4-D DMA**
Report:	Backus and Crosby, 1992c
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 nontarget phytotoxicity of 2,4-D DMA 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 rate schedules selected for the definitive tests were (rounded values) 1080, 540, 270, 135, 67.5, 33.8 and 16.8 g ae/ha for both the *in vitro* Petri dish germination component and the seedling emergence component. In addition, for the germination component, a further 5 rates were tested, namely 7.8, 4.5, 2.25, 1.13 and 0.56 g ae/ha. It seems that mustard and radish were not exposed to the four highest concentrations in the germination study, and were the only species exposed to the lowest four concentrations in this later study. There were three replicates of each of the treatments, including the untreated controls.

A volume of 2.0 mL of each treatment concentration was pipetted and evenly distributed onto the filter papers in each of the Petri dishes used for each treatment. Water was then pipetted onto the treated filter paper to bring the total volume of water in each dish to that volume predetermined to be optimal for each species. Ten 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 6.0, CEC of 3.4 meq/100 g and organic matter of 1.0%. 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. Application was to the soil surface using a moving laboratory sprayer.

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 to be 90% or more of the nominal values for both Petri dish and soil components of the study.

   Seed Germination: In general, the test material showed little activity on seed germination. Corn, cucumber, oats, onion, sorghum and soybean all had NOELs, EC25s and EC50s greater than the maximum dose of 1080 g ae/ha. The NOEL for buckwheat was 560 g ae/ha. Tomato was the only species exhibiting a dose response; a quadratic model was fitted to the data. Calculated NOEL, EC25 and EC50 values for this species were 270, 294 and 627 g ae/ha respectively.

Mustard and radish were exposed to a maximum dose of 67.5 g ae/ha because of results of the preliminary test. These species were insensitive to 2,4-D DMA at this level and the NOEL was ≥67.5 g ae/ha.

In the preliminary test, plants were exposed to rates of 6.7, 0.67 and 0.07 g ae/ha. Observations from this study showed that radicle length was influenced by 2,4-D DMA, yet this end point was not measured in the definitive test. With respect to impacts on radicle length in the preliminary study, corn seemed to be the least-sensitive species with a NOEL ≥6.7 g ae/ha. The NOEL for buckwheat, oats, onion, sorghum and soybean was 0.67 g ae/ha. The lowest rate tested, 0.07 g ae/ha, appeared to be the NOEL for cucumber, mustard and tomato. At this rate, radish continued to exhibit 45% inhibition and a NOEL was not determined. For percent germination, mustard and radish were adversely affected at 6.7 g ae/ha, although this level was not replicated in the definitive study.

Visual Observations: Fourteen days after application, the plants were harvested. However, visual observations were made just prior to this. All species in the untreated controls appeared normal, with emergence at least 85%. At the highest tested rate, no symptoms were observed on two of the four monocot species, corn and oats, this appeared to be the visual NOEL for these species. The remaining 8 species exhibited stunting of various degrees. Observations at 540 g ae/ha were similar with soybean occasionally appearing distorted.

At 270 g ae/ha soybean and tomato appeared normal and this was deemed the visual NOEL for these species. At this rate, stunting of various degrees was observed on buckwheat, cucumber, mustard, onion, radish and sorghum. Cucumber, mustard and radish exhibited stunting at 135 g ae/ha with this rate being the visual NOEL for buckwheat. The same species were the only species to exhibit effects at 67.5 g ae/ha. At 33.6 g ae/ha only cucumber appeared slightly stunted and this rate was deemed the visual NOEL for mustard and radish. At the lowest rate, 16.8 g ae/ha, only cucumber had any symptoms, and they appeared questionable.

Seedling Emergence

Emergence and Survival

Only mustard, onion and radish showed significant emergence effects in the test. The other species showed no significant effect at any dose. The most sensitive species was mustard, which had a significant linear dose response. Emergence was variable for mustard at test concentrations of 270 g ae/ha and up.

The results of this test were generally in agreement with the Petri dish test with all species showing no effect in the soil bioassay (except tomato) also showing no effect in the Petri dish assay. However, mustard and radish were affected in the soil at rates higher than those tested in the Petri dish for these species. Onion appeared more sensitive in the seedling emergence study compared to the seed germination study.

Fresh weight

Sorghum was the most sensitive monocot with a NOEL of 33.6 g ae/ha. Other monocots were relatively insensitive with onion the next most sensitive (NOEL of 540 g ae/ha and corn and oats not being affected at the highest test rate of 1080 g ae/ha. The EC25 and NOEL for onion, however, could be lower as one replicate at 540 g ae/ha exhibited 0% emergence and was removed from the data set for statistical analysis.

Mustard was the most sensitive dicot and exhibited a significant linear dose response. The estimated NOEL was at best, the lowest rate tested of 16.8 g ae/ha as was the EC25. Cucumber was the second most sensitive dicot, again with a NOEL at best equal to the lowest treatment rate and a calculated EC25 of 22.3 g ae/ha. The cucumber NOEL may have been higher than 16.8 g ae/ha; results at the next two levels were not significantly different to control values. However, fresh weight at 16.8 g ae/ha was significantly less than control values. Additionally, in the preliminary study, the NOEL for both cucumber and mustard was 6.7 g ae/ha. With soybean, fresh weight inhibition was 25% at 1080 g ae/ha. While this level was set to be the best case NOEL, variances for soybean were low and the study report indicates the NOEL could be considered to be 540 g ae/ha.

Statistical Results

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

Table A3.63: Effects of 2,4-D DMA on Seedling Emergence and Germination 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	≥1080	≥1080	≥1080	540	932[1]	≥1080	540	≥540	605
	Sorghum	≥1080	≥1080	≥1080	≥1080	≥1080	≥1080	33.6	50.7[4]	182[5]
	Corn	≥1080	≥1080	≥1080	≥1080	≥1080	≥1080	≥1080	≥1080	≥1080
	Oats	≥1080	≥1080	≥1080	≥1080	≥1080	≥1080	≥1080	≥1080	≥1080
Dicots	Tomato	270	294	627	≥1080	≥1080	≥1080	≥1080	≥1080	≥1080
	Soybean	≥1080	≥1080	≥1080	≥1080	≥1080	≥1080	≥1080	≥1080	≥1080
	Buckwheat	540	1080	≥1080	≥1080	≥1080	≥1080	270	307	625
	Mustard	≥1080	≥1080	≥1080	135	135-270	551[2]	≤16.8	≤16.8	39.2[3]
	Radish	≥1080	≥1080	≥1080	540	540-1080	≥1080	16.8	33.6	135
	Cucumber	≥1080	≥1080	≥1080	≥1080	≥1080	≥1080	≤16.8	22.3	495

Reported confidence limits in g ae/ha are: **1)** 342-20659; **2)** 231-6360; **3)** 17.7-64.8; **4)** 27.9-77.5; **5)** 123-285

Conclusions:

The seedling emergence in soil fresh weight results contrast with the seedling emergence results. For instance, buckwheat, cucumber, mustard, radish and sorghum have lower NOEL, EC25 and EC50 values for fresh weight inhibition than for emergence percent. This indicates that 2,4-D DMA can inhibit seedling growth of plants that emerge from treated soil. This demonstrates that the effect of 2,4-D DMA occurs on an active growth process.

The results of these tests indicate that mustard and radish are sensitive to 2,4-D DMA. Monocots such as corn and oats are virtually unaffected by the test material and the other monocot and dicot species are affected in an intermediate way, usually in a linear or quadratic dose response manner.

It is uncertain why the observation of sensitivity of radicle length as an indicator of adverse effects in the preliminary study was not followed up in the definitive study.

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 DEA salt 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 A3.64: Additional Seedling Emergence Results as Reported in US EPA, 2005

Species	NOEL – g ae/ha	EC25– g ae/ha	Endpoint affected
2,4-D DEA			
Monocot – Corn	840	1479	Fresh weight
Monocot - Onion	303	426	Dry weight
Monocot - Oats	420	1680	Fresh weight
Monocot - Sorghum	840	1132	Fresh weight
Dicot - Root Crop (Radish)	101	231	Emergence
Dicot - Soybean	213		Fresh weight
Dicot -Cucumber	213	235	Fresh weight
Dicot - Mustard	<50	50	Fresh weight
Dicot - Buckwheat	<50	50	Fresh weight
Dicot - Tomato	504	420	Emergence

The US EPA reports these rates in terms of "aei/Acre". It is unclear what this means. 2,4-D DEA salt has an acid equivalence of around 50.2%.

Table A3.65: Additional Vegetative Vigour Results as Reported in US EPA, 2005.

Species	NOEL – g ae/ha	EC25– g ae/ha	Endpoint affected
2,4-D DEA			
Monocot – Corn	840	>1680	Fresh weight
Monocot - Sorghum	213	258	Fresh weight
Monocot – Oat	>1680	>1680	Fresh weight
Monocot - Onion	11.2	44.8	Fresh weight
Dicot - Root Crop (Radish)	2.8	11.2	Fresh weight
Dicot - Soybean	5.6	50.4	Fresh weight
Dicot - Tomato	2.2	3.4	Fresh weight
Dicot - Buckwheat	2.8	43.7	Fresh weight
Dicot - Mustard	5.6	11.2	Fresh weight
Dicot - Cucumber	<2.8	9.0	Fresh weight

The US EPA reports these rates in terms of "ai/Acre". In terms of acid equivalence then, these rates will be halved. In their Reregistration Eligibility Document for 2,4-D, the US EPA reports mustard as being the most sensitive dicot for seedling emergence studies with acid/salt forms of 2,4-D with an EC25 of 50 g ae/ha, and tomato as being the most sensitive dicot for vegetative vigour testing of acid/salt forms of 2,4-D with an EC25 of 3.4 g ae/ha. Therefore, it appears that the values described above in terms of ae/ha are correct.

Conclusions for Terrestrial Toxicity

2,4-D DMA was slightly toxic to bees through either contact or oral exposure routes with LD50 values of >83.3 and 78 μg/bee respectively. Non standard tests using 2,4-D DMA and 2,4-D sodium salt to an Indian worker bee showed these chemicals to have an LD50 of 0.14% v/v and >3% w/v respectively, but these values 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 salts.

2,4-D DMA salt was harmless to a range of non-target terrestrial arthropods up to the highest rates tested, which in all cases was at least 1000 g ae/ha. Similarly, earthworms were not affected at rates up to 500 mg ae/kg soil when exposed to 2,4-D

DMA salt, making this chemical at worst, slightly toxic to earthworms. 2,4-D DMA salt was shown to have no significant impact on soil respiration or ammonification and nitrification of soil when applied at a rate up to 13.3 mg ae/kg soil. No other salt form of 2,4-D was tested on any other beneficial insect or soil micro-organisms.

Non-target terrestrial plant toxicity data (limited information only) indicate monocots were not as sensitive as dicots. Preliminary results in seedling emergence testing (2,4-D DMA) indicate that radicle length was the most sensitive indicator. No vegetative vigour studies were provided for any 2,4-D salt. However, non-assessed values for 2,4-D DEA indicate that dicots are much more sensitive than monocots with EC25 values based on fresh weight being <50 g/ha.