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 I- 2,4-D Acid

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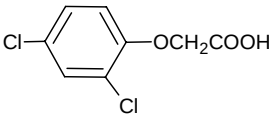
Australian Pesticides &
Veterinary Medicines Authority

Canberra
Australia

APPENDIX I – Technical Report For 2,4 Dichlorophenoxyacetic Acid (2,4-D)

Table of Contents:

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

	Acid
Common name (ISO)	2,4-D
Chemical name (IUPAC)	(2,4-dichlorophenoxy) acetic acid
Chemical name (CA)	acetic acid, (2,4-dichlorophenoxy)
CAS No	94-75-7
EEC No	202-361-1
Minimum purity[1]	960 ± 15 g/kg As given by FAO specification
Molecular formula	$C_8H_6Cl_2O_3$
Molecular mass	221.0
Structural formula	
Melting point[1]	139.25°C
Boiling point[1]	not applicable.
Appearance[1]	white or off-white crystalline powder, slight phenolic odour.
Relative density[1]	bulk density: 0.66 g/mL tap density: 0.81 g/mL
Vapour pressure[2]	1.86×10^{-5} Pa at 25°C
Henry's law constant[3]	$3.54x10^{-8}$ atm.m^3/mol
Solubility in water[4]	pH 1 buffered: 311±4 mg/L at 25°C pH 5 buffered: 20031± 1149 mg/L at 25°C pH 5 unbuffered: 29934± 2957mg/L pH 7 buffered: 23180± 590 mg/L at 25°C pH 7 unbuffered: 44558± 674 mg/L pH 9 buffered: 34196± 1031 mg/L at 25°C pH 9 unbuffered: 41314± 335 mg/L
Partition co-efficient (log Kow)[5]	pH 1: logKow = 2.70 at 25°C pH 5: logKow = 0.18 at 25°C pH 7: logKow = -0.83 at 25°C pH 9: logKow = -1.01 at 25°C
Dissociation constant	pKa = 2.87 (reported in Potter, 1990).

1) EC, 2001; 2)Chakrabarti and Gennrich, 1987a; 3) HenryWin v3.10; 4) Hopkins, 1987a; 5) Bailey and Hopkins, 1987.

2,4-D has growth-regulating and herbicidal properties in broad-leaved plants. Because of its solubility, 2,4-D is rarely used in the form of the acid; commercial 2,4-D herbicide formulations consist of the more soluble forms (such as alkali salts, amine salts), or esters. These are combined with solvents, carriers, or surfactants and are marketed in the form of dusts, granules, emulsions, or oil and water solutions in a wide range of concentrations (WHO, 1989).

The chemical is a solid at room temperature and its vapour pressure is indicative of only very slight volatility based on the scale of Mensink *et al*, 1995. Similarly, the Henry's Law Constant, calculated from vapour pressure and solubility measurements, is also indicative of only very slight volatility from water bodies, also based on

Mensink *et al*, 1995. The high solubility of 2,4-D at environmentally relevant pH values corresponds to low octanol-water partition co-efficients suggesting the chemical will not bioconcentrate or bioaccumulate through the food chain. The reported dissociation constant for 2,4-D indicates it will be in its dissociated form throughout the environmental pH range.

Hydrolysis

Report: Creeger, 1989a.

Guidelines: US-EPA Subdivision N; 161-1

GLP: yes

Test System

The hydrolysis of uniformly ring-labeled ^{14}C-2,4-dichlorophenoxyacetic acid (2,4-D) was studied at a concentration of about 21 ppm in sterilised aqueous solutions buffered at pH 5, 7 and 9. The test solutions were incubated for a minimum of 30 days in the dark at a constant temperature of 24.9±0.1^{o}C. Samples of the test solutions were taken immediately after treatment and at various sampling intervals during the study. Samples were analysed by LSC for total radioactivity (material balance) and were analysed by HPLC equipped with both a UV detector and a radioactivity flow detector connected in series for identification of parent and hydrolysis products. TLC was used as a confirmatory analytical method.

Findings

Analysis of the aliquots of the test solutions showed 2,4-D did not hydrolyse at any pH during this study. The major potential hydrolysis product predicted was 2,4-dichlorophenol (2,4-DCP) but was not detected at any pH during the study. All radioactivity detected at all pH levels was identified only as parent compound.

The amount of initially applied 2,4-D recovered during the study ranged from 100.2±3.6% (pH 5), 98.7±2.0% (pH 7) and 97.0±3.0% (pH 9). Since the standard deviation and standard error (2-4%) involved in conducting the study was about as great as the variation in concentrations of the test substance during the study, statistically reliable linear regression and half-life calculations could not be derived from the data.

Conclusion

2,4-D is hydrolytically stable at environmentally relevant pH and temperature conditions.

Report: Creeger, 1989b.

Guidelines: 40 CFR 769.3500

GLP: yes

Test System

The hydrolysis of uniformly ring-labeled ^{14}C-2,4-D was studied at a concentration of about 21 ppm in sterilised aqueous solutions buffered at pH 3, 7 and 11. The test solutions were incubated for a minimum of 30 days in the dark at a constant temperature of 24.9±0.1^{o}C. Samples of the test solutions were taken immediately after treatment and at various sampling intervals during the study. Samples were

analysed by LSC for total radioactivity (material balance) and were analysed by HPLC equipped with both a UV detector and a radioactivity flow detector connected in series for identification of parent and hydrolysis products. TLC was used as a confirmatory analytical method.

Findings

Analysis of the aliquots of the test solutions showed 2,4-D did not hydrolyse at any pH during this study. The major potential hydrolysis product predicted was 2,4-dichlorophenol (2,4-DCP) but was not detected at any pH during the study. All radioactivity detected at all pH levels was identified only as parent compound.

The amount of initially applied 2,4-D recovered during the study ranged from 99.5±5.1% (pH 3), 98.7±2.0% (pH 7) and 97.2±2.8% (pH 11). Since the standard deviation and standard error (2-4%) involved in conducting the study was about as great as the variation in concentrations of the test substance during the study, statistically reliable linear regression and half-life calculations could not be derived from the data.

Conclusion

The outcomes of this study confirm those of Creeger 1989a and further demonstrate that 2,4-D is hydrolytically stable beyond environmentally relevant pH and temperature conditions.

Photodegradation in Water

Report: Creeger, 1989c.

Guidelines: US-EPA Subdivision N; 161-2

GLP: yes

Test System

The aqueous photodegradation of uniformly ring-labelled ^{14}C-2,4-D was studied at a concentration of about 5 ppm in a filter-sterilised aqueous solution buffered at pH 7. The test solution was irradiated for 30 days under simulated sunlight on an approximately 12:12 hour light:dark cycle at 24.8±0.7^{o}C. The emission spectrum from the xenon burner was calibrated to simulate natural sunlight between 290 and 750 nm and the spectrum was shown to approximate that for natural sunlight in Phoenix, Arizona (although the time of the year was not specified).

The reaction vessel containing the test solution was connected to a series of traps for the collection of radioactive carbon dioxide and organic volatiles that were produced. Humidified air was used to sweep the volatiles into the trapping solutions. Samples of the test solution were taken immediately after treatment and at various sampling intervals during the study. Duplicate aliquots of the test solution samples were analysed for total radioactivity by LSC. Additional duplicate aliquots were analysed by HPLC equipped with both a UV detector and a radioactive flow detector connected in series for the identification of 2,4-D and any photodegradation products. The trapping solutions were replaced at each sampling interval, and duplicate aliquots of each trapping solution were assayed by LSC to determine total radioactivity. TLC was used as a confirmatory analytical method.

Findings

Table A1.1 shows the degradation behaviour of 2,4-D during the aqueous photolysis study. The half-life under these conditions was calculated to be 12.98 calendar days (7.57 days of constant light exposure). A dark control study at pH 7 showed no degradation of the test substance.

Table A1.1 – Aqueous Photolysis of 2,4-D and Formation of its Photodegradation Products[1]

Time (days)	2,4-D	1,2,4-benzenetriol	Unknown polar products[2]	Unknown polar products[3]	$^{14}CO_2$	Total
0	100.0	0.0	0.0	0.0	0.0	100.0
1	93.5	0.4	0.9	1.3	0.3	96.4
7	69.3	7.8	5.0	8.3	2.5	92.9
9	61.1	10.0	6.3	9.7	4.3	91.4
11	54.8	13.7	8.1	11.1	6.0	93.7
12	53.2	15.2	9.0	10.1	7.6	95.1
16	40.0	21.1	9.8	10.7	11.6	93.2
30	21.3	37.7	12.7	9.1	25.0	105.8

1. Based on the result of the hydrolysis tests, no degradation products observed are attributed to hydrolysis.
2. This region on the developed TLC plates was composed of numerous polar, minor products of which no single product exceeded 10% of the level of radioactivity in the test solution.
3. This region on the developed TLC plates was composed of numerous polar, minor products of which no single product exceeded 10% of the level of radioactivity in the test solution.

The only photodegradation product present in the irradiated samples at >10% applied radioactivity (AR) was identified as 1,2,4-benzenetriol. Formation of this photodegradate does not appear to have peaked by the end of the study. Production of $^{14}CO_2$ reached 25% at the end of the study.

The overall material balance during the course of the study was 96.8±2.4% AR.

Conclusion

Photodegradation from water may be a factor in the removal of 2,4-D in the environment. The half-life calculated was around 13 days indicating this chemical would not persist in clear aqueous solution.

Report: Klöpffer, 1991

Guidelines: UBA Test Guideline Direct Phototransformation

GLP: yes

Test System

The direct phototransformation of 2,4-D was determined in water following UBA Guidelines. The substance was dissolved in water and the decrease in concentration under illumination with UV-radiation at 304 nm with time was followed by HPLC. The wavelength used was a compromise between the absorption intensity (too low at 313 nm) and the availability of a suitable mercury line of the lamp. The intensity of the UV-radiation (spectral photon irradiance) was measured using chemical actinometry. The quantum efficiency of the disappearance was calculated for the irradiation wavelength.

Findings

2,4-D was stable in water for a period of 7 days at 25-30^{o}C (room temperature) and exposed at day light. The concentration (ppm) was 2.34 and 2.38 at days 0 and 7 respectively.

From the upper limit of quantum efficiency a lower limit of the environmental half-life in surface water was calculated according to the Battelle-UBA computer program. The environmental conditions are those of average spectral photon irradiance for April and May (Central Europe). With regard to the surface water two conditions were used:

1. 1 cm distilled water as a model for optimal irradiance (no absorption except from the test substance);
2. 100 cm river water, using actual measured UV-absorption data from the river (Neckar (Germany); these data as well as the irradiance data are a part of the computer model.

The initial concentration used for the calculations is the concentration used in the irradiation experiment.

The average half-life in days for the 1 cm distilled water samples were $\geq 2.1X10^{3}$ for April and $\geq 1.1X10^{3}$ for May, with corresponding values for the 100 cm river water samples of $\geq 3.9X10^{4}$ and $\geq 2.4X10^{4}$ respectively.

Conclusion

Direct phototransformation in water is unlikely to be a significant removal process of 2,4-D in the environment.

Photodegradation in Soil

Report: Creeger, 1989d

Guidelines: US-EPA Subdivision N; 161-3

GLP: yes

Test System

The soil photodegradation of uniformly ring-labelled ^{14}C-2,4-D mixed with non-labelled 2,4-D was studied at a concentration of 4.35 ppm on air-dried, sieved (2 mm) and autoclave-sterilised loam soil. The test soil was irradiated for 30 days under simulated sunlight on a 12:12 hour light:dark cycle. The emission spectrum from the xenon burner was calibrated to simulate natural sunlight and the spectrum was shown to approximate that for natural sunlight in Phoenix, Arizona (although the time of the year was not specified). The temperature of the test soil was maintained at 24.9±0.8^{o}C during the study. The soil was continuously purged with air to flush any volatile products formed into a series of trapping solutions (0.2M sodium hydroxide solution, ethylene glycol and 1M sulfuric acid). In addition, aliquots of the prepared soil were treated with 2,4-D at 4.31 ppm and incubated in the dark at 24.9±0.1^{o}C, to serve as dark control samples.

Samples of the irradiated test soil were taken immediately after treatment and at various sampling intervals during the study. Duplicate aliquots of the test soil samples were combusted and analysed for total radioactivity by LSC. Additional

duplicate aliquots were extracted with ether and the extracts analysed by LSC. The ether extracts were concentrated and analysed by HPLC equipped with both a UV detector and a radioactive flow detector connected in series for identification of the 2,4-D and any soil photodegradation product that was formed at a level ≥10% AR. TLC was used as a confirmatory analytical method.

Findings

Table A1.2 shows the distribution of radioactivity in the irradiated soil samples.

Table A1.2: Distribution of Radioactivity (% AR)

	Irradiation time in days (hours)					
Recovery of Radioactivity[1]	0 (0)	2 (26.0)	8 (107.6)	13 (174.8)	21 (274.8)	30 (381.4)
Extractable Radioactivity						
Ethyl Ether Extract[2]	98.79	87.40	89.23	88.98	93.76	85.71
Acid Aqueous Extract	0.54	0.42	7.16	3.23	4.90	0.34
Sodium Hydroxide Extract	0.45	0.61	0.07	2.49	4.80	1.34
Unextractable Radioactivity	0.22	0.40	0.15	0.43	0.99	0.42
Cumulative Volatiles[3]						
$^{14}CO_2$ (0.2M NaOH)	NA[4]	0.38	0.83	1.71	3.59	5.05
1M H_2SO_4 Trap	NA	0.05	0.49	0.49	1.3	1.32
Ethylene Glycol Trap	NA	ND[5]	ND	ND	ND	ND
Total Radioactivity Recovered	100.00	89.26	97.93	97.93	109.34	94.18

1. Average of four measurements at each sampling time
2. HPLC analysis of all ether extracts showed 2,4-D to be the only compound present
3. Average of two measurements at each sampling
4. Not analysed
5. Not detected, detection limit of 1.1% (0.05 ppm)

Analysis of irradiated and dark control samples showed that the chemical did not undergo substantial photodegradation in the irradiated soils. No degradation occurred in the dark control samples. During the study there were no compounds in the test soil at levels of 10% AR or more other than parent compound.

At no time during the study did any other extract or trapping solution contain more than 7.2% AR. HPLC analysis indicated radioactivity in other soil extracts did not consist of a single compound but a mixture of many minor compounds each less than 1.1% AR.

CO_2 production reached 5.05% AR by the end of the study.

The material balance during the study in irradiated samples ranged from 89.26-109.34% (confirmed by combustion and LSC analysis of soil prior to extraction which showed a material balance of 85.62-102.65%).

Conclusion

The data indicate 2,4-D is stable to soil photodegradation with a theoretically calculated half-life of 68 calendar days using linear regression. However, the correlation coefficient (r^2) of –0.4031 indicates degradation does not follow first-order kinetics in this study, and the calculated half-life is not statistically reliable and should be treated with caution.

Degradation in Soil and Water

Soils – Aerobic

Report: Concha and Shepler, 1994a

Guidelines: US-EPA Subdivision N; 161-1

GLP: yes

Test System

An aerobic soil metabolism study of ^{14}C-2,4-D, uniformly labelled in the phenyl ring, in viable Carlin silty clay soil was conducted at an application rate of 5.1 ppm for up to 16 days. The soil characteristics were as follows:

Table A1.3: Soil characteristics of Carlin Silty Clay.

Texture	Bulk Density	% Sand	% Silt	% Clay	OM (%)	CEC (meq/100 g)	pH	WHC[1]
Silty clay loam	1.24 g/cc	11.2	60.0	28.8	3.87	10.08	6.9	27.16

1) % at 1/3 Bar.

The test systems were prepared by adding the equivalent of 50 g dry weight of soil (56 g) to biometer flasks. Some of the flasks were dosed at the same rate (5.1 ppm) but using 59.3 g dry weight equivalent of soil (66.4 g). Dosing solution was added in water to achieve 75% field moisture capacity (4.2 mL and 5.0 mL respectively). The average temperature was 25^{o}C. Sampling was conducted at days 0, 1, 2, 3, 5, 7, 9, 13 and 16.

A polyurethane foam plug was used to trap organic volatiles and 10% KOH trapping solution (50 mL) was used for capturing CO_2.

Soil was extracted three times (1 X 100 mL, 2 X 50 mL) with acetone:water:acetic acid, 90:5:5 (v:v:v), and the extracts combined. LSC was used to determine radiocarbon. HPLC was used to identify the parent and degradates. HPLC and TLC were used for confirmation of the degradates.

Samples were extracted and radioassayed immediately after sampling. All samples and reference standards were stored frozen when not in use. A sample extract analysed after 80 days under storage conditions showed no further degradation.

Findings

2,4-D degraded rapidly in the Catlin soil and represented 0.5% AR after 16 days exposure. The calculated half-life was 1.7 days based on pseudo first order kinetics using Day 0-9 data (r^2 = 0.98). Table A1.6 shows product distribution and recoveries of radiocarbon (average percentages).

Table A1.6: Average Product Distribution and Radiocarbon Recovery (% AR)

Day	2,4-D	2,4-DCP	2,4-DCA	Others[1]	Bound	CO_2	Organic volatiles[2]	TOTAL
0	89.0	0.0	0.0	1.1	5.0	0.0	0.0	95.1
1	74.3	2.7	0.1	0.6	12.4	0.8	0.0	90.9
2	65.5	3.5	0.5	0.4	20.4	3.8	0.0	94.1
3	40.3	2.7	0.7	0.6	44.3	15.6	0.1	104.3
5	12.9	2.0	1.4	0.4	52.8	37.2	0.1	106.8
7	6.4	1.9	1.5	0.5	39.2	42.6	0.2	92.3
9	2.6	1.5	2.7	0.5	42.3	44.7	0.1	94.4
13	1.0	0.8	2.5	0.3	39.3	49.3	0.2	93.4
16	0.5	0.4	1.5	0.8	35.8	51.2	0.3	90.5

1) This category is comprised of several bands by HPLC, each at <0.2% AR. A peak eluting at around 38.6 min (HPLC) rose to 0.25% AR at day 9 and declined to around 0.1% AR.
2) HPLC analysis of the day 16 replicate (B) foam plug extract showed that the radioactivity recovered in the plug was primarily due to 2,4-DCA.

The major metabolite was CO_2 which comprised 51.2% AR at the end of the study period. Two major soil metabolites, 2,4-dichlorophenol (2,4-DCP) and 2,4-dichloroanisole (2,4-DCA) were present in the soil extracts at maximum concentrations of 3.5% (day 2) and 2.5% (day 9) respectively. The unextractable residue comprised 35.8% at the end of the study. Fulvic acid/humic acid partition of an extracted residue sample afforded 16.1% AR in the fulvic acid fraction and 11.1% in the humic acid fraction. Further HPLC analysis of the fulvic acid fraction showed 6.1% AR was recovered as 2,4-D.

Conclusion

Results indicate that 2,4-D and its soil degradates should dissipate rapidly from the soil environment, with mineralisation and incorporation, partly unchanged, into the soil organic matter as the principal pathways of carbon disposition.

Report: Cohen, 1990a

Guidelines: US-EPA Subdivision N; 161-1

GLP: yes

Test System

An aerobic soil metabolism study of ^{14}C-2,4-D, uniformly labelled in the phenyl ring, in viable Mississippi silt loam soil was conducted at an application rate of 3.69 ppm. The soil characteristics were as follows:

Table A1.7: Soil characteristics of Carlin Silty Clay.

Texture	Bulk Density	% Sand	% Silt	% Clay	OM (%)	CEC (meq/100 g)	pH	WHC
Silty clay loam	Not given	26	60.0	14	1.4	9.8	7.4	Not given

The soil was sieved (2-mm mesh) and non-sterilised. Untreated soil was pre-incubated under aerobic soil conditions in darkness at 24.9±0.4°C for 5 days to activate soil microbes. Following the activation period, ^{14}C-2,4-D in acetonitrile was added to the soil in the incubation flask and mixed thoroughly, resulting in a soil concentration of 3.69 ppm. The test soil was then aerobically incubated in darkness at 24.9±0.4°C for 56 days. The test soil was continuously purged with humidified air at

25-30 mL/minute to flush any volatile compounds formed into a series of trapping solutions (ethylene glycol, 1M sulfuric acid, 5% sodium hydroxide and Carbosorb).

Samples of the soil were taken immediately after treatment (Day 0), and at 2, 4, 7, 15, 24, 35 and 56 days after treatment. Quadruplicate test soil aliquots were combusted and analysed for total radioactivity by LSC. Additional duplicate samples were extracted with 1.5 M phosphoric acid mixed with ethyl ether, washed with water, and finally re-extracted with 1M sodium hydroxide solution. Aliquots of all extracts were analysed by LSC. Duplicate aliquots of the trapping solutions were analysed directly by LSC.

The soil extracts were analysed by HPLC equipped with both a UV (280 nm) detector and a radioactive flow detector connected in series. Radioactive flow detection allowed for the identification of 2,4-D acid and its aerobic soil metabolites that were formed at a level ≥0.01 ppm. One-dimensional TLC was used as a confirmatory analytical method for the identification of 2,4-D acid and its aerobic soil metabolites.

Findings

The rate of 2,4-D degradation was significantly less rapid than that found in Concha and Shepler (1994) above. The calculated half-life was 66.2 calendar days based on pseudo first order kinetics that was longer than the incubation period. Table A1.8 shows product distribution and recoveries of radiocarbon (average percentages).

Table A1.8: Average Product Distribution and Radiocarbon Recovery (% AR)

	Extractable residues[1]				Volatiles[2]		
Day	2,4-D	Others	Total Extractable	Bound	CO_2	Organic volatiles[2]	TOTAL
0	96.8	2.5	99.3	2.6	NA[3]	ND	101.9
2	81.0	1.5	82.5	8.7	1.7	ND	92.9
4	81.6	1.5	83.1	9.7	2.6	ND	95.4
7	88.2	1.2	89.4	11.1	3.6	ND	104.4
15	66.3	1.6	67.9	21.0	7.5	ND	96.4
24	72.9	3.3	76.2	14.4	10.3	ND	100.9
35	62.6	2.5	65.1	14.1	12.7	ND	91.9
56	54.6	7.8	62.4	20.3	19.7	ND	102.4

1) Total extractable residue is derived from LSC. HPLC analysis for each time interval determined the level of 2,4-D. "Others" is the difference. None of the metabolites tested for were observed. Average of 4 measurements at each sampling time.
2) Average of two measurements at each sampling time.
3) Not analysed

Analysis of soil extracts showed 2,4-D acid represented 54.6% AR and carbon dioxide was the major metabolite formed during the 56 day aerobic incubation period. All ^{14}C-residues ≥0.01 ppm were identified during this study.

Based on the list of metabolites predicted, and standards received for analysis during the test, the following chemicals appear to have been tested for (other than parent compound): 2,4-DCP, phenoxyacetic acid, *o*-chlorophenoxyacetic acid, *p*-chlorophenoxyacetic acid, chlorohydroquinone (1,4-dihydroxy-2-chlorophenol), 1,2,4-benzenetriol, 2 chlorophenol and 4-chlorophenol. None of these appear to have been detected.

CO_2 production reached 19.7% AR by Day 56. No other residues greater than 0.01 ppm were observed. The extraction recovery of the soil samples ranged from 88.4 - 108.2% AR at each sampling interval (as determined by soil combustion). The

material balance during the aerobic soil incubation period (soil extractable and unextractable + cumulative volatiles) ranged from 92.0 – 104.3% AR.

Conclusion:

Linear regression analysis of the data indicates that 2,4-D acid was metabolised with a half-life of 66.2 calendar days. The correlation coefficient (r^2) was 0.8313.

The results of this study are at odds with the Concha and Shepler, 1994a study above where metabolism in aerobic soils was significantly faster. The reasons for this are unclear, but rapid metabolism in aerobic soils is supported by the aged residue in column leaching studies (see further below), where extensive metabolism of 2,4-D occurred over the 28-30 day ageing period.

Soils – Anaerobic

No studies were provided for this endpoint.

Water – Aerobic

Report: Cohen, 1991a

Guidelines: US-EPA Subdivision N; 162-4

GLP: yes

Test System

The aerobic aquatic metabolism of uniformly ring-labelled ^{14}C-2,4-D was studied at a concentration of 4.63 ppm in sieved (2-mm), non-sterilised Louisiana rice paddy sediment (clay soil) and water mixture. The sediment characteristics were as follows:

Table A1.9 Chemical and physical properties of sediment.

Parameters		Louisiana rice paddy	
Textural Class		Clay	
Sand [%]		8	
Silt [%]		24	
Clay [%]		68	
pH		7.3	
Bulk Density (g/cm^3)		1.20	
Organic Matter (%)		3.6	
Cation Exchange Capacity [meqN/100 g dry sediment]		28.9	
		Before*	End
Redox Potential (mV)		-	-
Biomass (CFU/g)	Bacteria	$2.14X10^5$	-
	Actinomycetes	$4.81X10^4$	-
	Fungi	$1.1X10^3$	-

* following the end of the 281 day activation period.

The only water characteristic provided was the pH of 8.03. The protocol states that at least pH and total hardness should be provided. Additionally, for the definitive study, the protocol states that the dissolved oxygen content or redox potential of the water will be determined at the end of the pre-incubation period to demonstrate the aerobicity of the water/sediment system. These measurements do not appear in the report.

Untreated sediment/water mixture (consisting of 320.4 g sediment and 533.9 mL water) was pre-incubated under aerobic aquatic conditions in darkness at 24.7±0.3°C for 218 days to activate microbes. Following the activation period, ^{14}C-2,4-D in

acetonitrile was added to the sediment/water mixture in the incubation flask and mixed thoroughly, resulting in a test system concentration of 4.63 ppm. The test system was then aerobically incubated in darkness at 24.7±0.3°C for 30 days. The test soil was continuously purged with humidified air at 30 mL/minute to flush any volatile compounds formed into a series of trapping solutions (ethylene glycol, 1M sulfuric acid and 5% sodium hydroxide).

Upon application of the test substance, the water/sediment mixture was blended on low speed for around 1 minute to ensure homogenous distribution among the system. Samples of the test system were taken immediately after treatment and at 2, 5, 12, 20, 27 and 30 days after treatment. Test sediment/water samples were separated by centrifugation and test sediment aliquots were combusted and analysed for total radioactivity by LSC. The remaining sediment samples were extracted with 1.5M phosphoric acid mixed with ethyl ether, washed with water, and finally re-extracted with 1N sodium hydroxide solution. Aliquots of all extracts were analysed by LSC. Duplicate water supernatant samples and trapping solutions were analysed by LSC.

The water supernatant and sediment extracts were analysed by HPLC equipped with both a UV (280 nm) detector and a radioactive flow detector connected in series. Radioactive flow detection allowed for the identification of 2,4-D and its aerobic aquatic metabolites that were formed at a level ≥0.01 ppm (LOD of 0.005 ppm). One dimensional TLC was used as a confirmatory analytical method for the identification of 2,4-D and its aerobic aquatic metabolites.

Findings

Initially, around 60 and 40% AR was found in the water and sediment respectively in the form of 2,4-D. These declined throughout the test period, and residue distribution in terms of % AR is summarised in Table A1.10 below.

Table A1.10 Proportion of radioactive components (% AR) in water and sediment.

DAT	2,4-D	CHQ	2,4-DCP	Unknowns[1]	Bound	TOTAL
Water						
0	56.3	ND[2]	ND	2.7	-	59.0
2	52.7	1.0	ND	1.4	-	55.1
5	49.4	1.1	ND	1.6	-	52.1
12	46.7	1.0	ND	1.8	-	49.5
20	42.6	1.3	ND	2.9	-	46.8
27	5.4	16.4	ND	1.8	-	23.6
30	2.3	10.7	ND	1.9	-	14.9
Sediment						
0	37.6	ND	ND	2.7	0.7	41.0
2	36.4	ND	ND	2.6	0.9	39.9
5	40.0	0.5	ND	2.8	1.6	44.9
12	41.5	0.3	0.6	3.8	2.0	48.2
20	32.2	0.6	0.3	3.3	3.6	40.0
27	15.5	0.5	ND	6.4	16.1	38.5
30	6.4	0.4	4.9	7.1	18.7	37.5

1) Unknowns are based on total radioactivity found through LSC less the amount of 2,4-D, CHQ and 2,4-DCP found through HPLC analysis.
2) ND = not detected

The metabolite, chlorohydroquinone (CHQ), reached a maximum concentration of 16.9% AR on Day 27 and declined to 11.1% AR by Day 30. A minor metabolite, 2,4-

DCP, accounted for 4.9% AR by Day 30. CO_2 production reached 15.9% AR by Day 30.

The report provides a linear regression analysis of the total radioactivity data and calculates a half-life of 15 days with a first order rate constant of –0.0667. DEH, upon re-plotting these data, determines the same rate constant, r^2 value and regression equation constant as that found in the study. However, the half-life obtained from this regression is 10.4 days. Corresponding half-lives for water and sediment (as plotted by DEH) equate to 7.4 and 14.6 days respectively.

The plots for concentration vs time appear biphasic in nature. For the first 20 days, elimination is relatively small with 76% AR still being found as parent compound at 20 days. Correspondingly, only limited CO_2 (1.6% AR) is found at this time. The total system half-life estimated for the first 20 days is around 71.5 days. From days 20-30, a significant amount of CO_2 is produced compared to the first phase with almost 16% recorded at day 30. Also during this time, there is significant removal of parent compound from the system with only 8.7% AR found by day 30. The half-life for this second phase is estimated to be 3.3 days (based on 3 data points only).

The material balance during the study period (soil extractable and unextractable + water soluble + cumulative volatiles) ranged from 100.0% (day 0) to 68.8% AR (day 30). The decline in the material balance occurred after Day 20 and correlated with the production of CO_2 and the precipitous decrease in 2,4-D concentration in the test system. This infers that the material balance deficit at the later sampling intervals (71.3% AR at day 27 and 68.8% AR at day 30) was due to inefficient trapping of $^{14}CO_2$ in the sodium hydroxide trap. The author notes that this material loss does not affect the degradation rate of 2,4-D.

Conclusion:

Degradation of 2,4-D in the water/sediment system described above appears to be biphasic with slower degradation over the first 3 weeks (half-life estimated to be around 71.5 days) and significantly faster degradation from then on (half-life around 3.3 days).

Report:	Concha and Shepler, 1993a
Guidelines:	US-EPA Subdivision N; 161-4
GLP:	yes

Test System

An aerobic aquatic metabolism study of ^{4}C-2,4-D, uniformly labelled in the phenyl ring, in viable pond sediment and water from Henry County, Illinois was conducted at an application rate of 5 ppm for up to 46 days.

Table A1.11 Chemical and physical properties of sediment.

Parameters - Sediment			
Textural Class		Silty Clay Loam	
Sand [%]		8.0	
Silt [%]		54.4	
Clay [%]		37.6	
pH		7.3	
Bulk Density (g/cm^3)		1.18	
Organic Matter (%)		4.53	
WHC (%) @ 1/3 Bar		43.88	
Cation Exchange Capacity [meqN/100 g dry sediment]		18.47	
		Before*	Day 35
Redox Potential (mV)		+84	-22
Dissolved Oxygen (ppm)		6.17	6.05
Biomass (CFU/g)	Bacteria	$4.2X10^6$	$32.0X10^6$
	Actinomycetes	$1.7X10^6$	$6.25X10^6$
	Fungi	$0.022X10^6$	-
Parameters - Water			
Alkalinity (mg $CaCO_3$/L)		290	
Total Suspended Solids (mg/L)		136	
pH		6.9	
Hardness (mg $CaCO_3$/L)		374	
		Before*	Day 35
Biomass (CFU/g)	Bacteria	$0.16X10^6$	$0.0555X10^6$
	Actinomycetes	$0.0135X10^6$	No growth
	Fungi	$0.023X10^3$	-

- following the end of the 281 day activation period.

Tebbutt (1992) states that aerobic systems have redox potentials greater than +200 mV while anaerobic systems have redox potentials below +50 mV. The sediment redox potential values above show the sediments were marginally anaerobic at the start of the study, but by the end could be considered to be anaerobic in their nature. The test system was maintained at 25^{o}C and prepared by adding the equivalent of 20 g dry weight of sediment (62 g) and pond water (57 mL) to a total volume of 100 mL water to biometer flasks. Dosing solution (1 mL) was added in water. A polyurethane foam plug was used for trapping organic volatiles and 10% KOH trapping solution (50 mL) was used for trapping CO_2. Trap solutions and foam plugs were exchanged at weekly intervals and at sampling times.

Sampling was undertaken at Days 0, 4, 7, 11, 14, 20, 25, 35 and 46. Following separation of the sediment and water, the sediment was extracted once with acetone:water:1M NH_4OH, 90:5:5 (v:v:v) followed by an acidic extraction with two portions of acetone:water:acetic acid, 90:5:5 (v:v:v). No extraction was performed on the aqueous samples. LSC was used to determine radiocarbon. HPLC was used to identify the parent and degradates. HPLC and TLC were used for confirmation of degradates. Samples were extracted and radioassayed (LSC) immediately after sampling. Water samples were stored under refrigerated (<10^{o}C) conditions. All other samples and reference standards were stored frozen when not in use.

Findings

The pattern of parent breakdown and metabolite formation in the whole water/sediment system is shown below in Table A1.12.

Table A1.12: Average Product Distribution and Radiocarbon Recovery (% AR)

Day:	0	4	7	11	14	20	25	35	46
% 2,4-D	93.6	92.6	91.3	87.6	82.5	79.3	74.6	5.1	0.5
% 2,4-DCP	0.0	0.3	0.7	0.6	0.5	0.8	0.7	1.1	0.1
% 4-CPA	0.1	0.3	0.2	0.7	0.1	0.0	0.1	0.0	0.0
% 4-chlorophenol	0.0	0.0	0.2	0.7	1.2	1.4	1.3	0.5	0.0
% Unknown 1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	1.1
% Others*	2.7	0.1	0.7	1.8	1.9	0.9	2.5	0.6	2.8
% CO_2	0.0	0.1	0.2	0.5	0.9	3.3	6.6	54.0	63.9
% Organic volatiles	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0
% Bound	1.4	4.9	4.9	5.4	6.2	7.0	7.1	15.2	15.6
TOTAL	97.8	99.3	98.3	96.8	93.9	93.8	92.9	77.5	83.4

* This category is comprised of several bands by HPLC, each at <0.2% of the applied dose.

Given the very low formation of metabolites, only 2,4-D is reported below in terms of amounts in water and sediment:

Table A1.13: 2,4-D residues (% AR) dissipation from water and sediment:

DAT	Water	Sediment	Total
0	77.1	16.5	93.6
4	76.4	16.2	92.6
7	73.4	17.9	91.3
11	71.7	15.9	87.6
14	66.5	16.0	82.5
20	62.9	16.4	79.3
25	61.6	12.9	74.5
35	2.9	2.2	5.1
46	0.3	0.2	0.5

2,4-D degraded slowly in the first 25 days of exposure and still represented around 75% AR at 25 days. In the next 10 days, a rapid decrease in the amount of parent was observed and by study end (46 days), 2,4-D represented only 0.5% AR. This lag time was also observed in Cohen (1991a) and is postulated to be due to the time required for the microbial population to either produce the enzyme used in the breakdown of 2,4-D or to mutate in order to produce the specific enzyme. Based on this, the degradation data do not seem to follow first order kinetics. The authors report that extrapolation from the plot of time of exposure vs % 2,4-D shows the time when the concentration of parent reaches 50% is at approximately 29 days after application. When degradation in the pond water/sediment system was modelled using Monod-with-growth kinetics, the half-life was 4.5 days.

When DEH replotted the degradation data for 2,4-D using a biphasic pattern of residue decline, the first half-lives for water and the whole system (expected to occur over the first 3 to 4 weeks of exposure) were predicted to be 68.6 and 71.5 days respectively with the second phase half-lives significantly faster at 2.7 and 2.9 days respectively.

The major metabolite was CO_2, which comprised around 64% AR at the end of the study. 2,4-DCP, 4-CPA and 4-chlorophenol were present in both water and sediment extracts at low levels. 2,4-DCP represented a maximum of 1.1% AR at day 35. 4-CPA rose to 1.1% AR at day 14 before decreasing to untraceable amounts. 4-chlorophenol represented 1.4% AR after 20 days but was untraceable by the end of the study. One unknown was observed in the water phase of the samples after 35 days

and represented 1.1% AR at the end of the study. This unknown peak was unstable to frozen or refrigerated storage and converted to CO_2 and other volatile products upon acidification of the water phase. No organic volatiles were detected in levels >0.1%. The unextractable residue increased with time of exposure and comprised 16.5% AR at the end of the study.

Radiocarbon recoveries averaged 92.8±7.0% for the study. During the 25 d lag time, most of this (64%) was found in the water phase (see Table A1.13). Additionally, approximately 10-14% was extracted off the sediment using alkaline solvent, and about 4% was extractable in acidic acetone. After the lag time the radiocarbon distribution shifted dramatically as 2,4-D was degraded. At the end of the study period, 3% AR was recovered in the water phase, with 1.0% and 0.6% extracted into base and acidic solvents respectively. The overall material balance dropped slightly at day 35 but increased at day 46. This decline in mass balance is attributed to the rapid, massive production of CO_2 following lag period that may not have been efficiently trapped during the time of aeration and trapping solution changes. A supplemental sample set up as in the definitive study was incubated and left unopened for 39 days. The higher overall recovery obtained for this sample (92.4%) confirmed that the loss of radiocarbon in the definitive study was due to loss of CO_2 in the headspace not yet sequestered in the caustic trap.

Conclusion:

Degradation of 2,4-D in the water/sediment system described above appears to be biphasic with slower degradation over the first 3-4 weeks (half-life estimated to be around 71.5 days) and significantly faster degradation from then on (half-life around 2.9 days).

Report:	Fathulla, 1996a
Guidelines:	US-EPA Subdivision N; 162-4
GLP:	yes

Test System

The aerobic aquatic metabolism of 2,4-D, uniformly labelled in the phenyl ring, was studied in representative sediment and water from Lake Mendota, Dane County, Madison, Wisconsin. 18 samples were prepared by placing approximately 3 g (dry weight equivalent) of 2-mm sieved sediment and 30 mL of lake water in glass containers. The sediment and water had the following characteristics:

Table A1.14 Chemical and physical properties of sediment and water.

Parameters - Sediment		
Textural Class		
Sand [%]	98	
Silt [%]	2	
Clay [%]	0	
pH	8.8	
Bulk Density (g/cm^3)	Not given	
Organic Matter (%)	0.4	
WHC (%) @ 1/3 Bar	Not given	
Cation Exchange Capacity [meqN/100 g dry sediment]	8.4	
	Day 0	Day 35
Redox Potential (mV)[1]	358	90.3
Dissolved Oxygen (ppm)[1]	5.2	2.4
Parameters - Water		
Alkalinity (mg $CaCO_3$/L)	190	
Total Suspended Solids (ppm)	4	
Dissolved Oxygen (ppm)	6.3	
pH	8.46	
Conductivity (mmhos)	0.5	
Hardness (mg/L)	236	

1) assumed to be for the whole water/sediment sample slurry.

Unlike the above sediment/water system, this one shows sediments to be aerobic (redox > +200 mV based on Tebbutt, 1992) at the start of the study, and only marginally anaerobic by day 35 (with anaerobic conditions established at +50 mV and lower – Tebbutt, 1992).

A slurry of the lake water and sediment was analysed for the presence of microorganisms. Aerobic and anaerobic plate count assays were plated using agar and incubated at around 35^{0}C for around 48 hours. Plates were counted by visual observation using a manual plate reader with the following results:

Assay	Day 0	Day 30
Aerobic plate count	1.25 X 10^6	12.2 X 10^6
Anaerobic plate count	53 X 10^3	12.8 X 10^3

Plate count per mL of sediment/water slurry, expressed as colony forming units (CFUs)

The test material solution was applied to the water of each sample at a nominal concentration of 10 ppm. The sample containers were sealed and gently shaken, and the water level was marked on each container. The Day 0 samples were not placed under test conditions and were analysed as soon as possible after dosing. To maintain aerobic conditions and to collect volatile components, the remaining samples were connected to a glass manifold that was attached to a series of traps: ethylene glycol, 0.1 N sulfuric acid and two 2N KOH traps. Continuous air bubbling from the manifold to the traps was created by vacuum. The test apparatus was maintained in a dark, temperature-controlled room at 25^{0}C±1^o. Sample test conditions were measured by monitoring pH, oxidation-reduction potential and dissolved oxygen for all samples at the beginning and throughout the study in order to ensure that the aerobic conditions were maintained.

Duplicate samples were collected at Days 0, 1, 3, 8, 16, 23 and 30. At all intervals that samples were collected, the trapping media were also collected and replaced with fresh media. One sample was collected on Day 30 for microbial analysis to ensure that the samples were kept viable during the study.

The sample water layer and sediment were separated by centrifugation. The concentration of radioactivity in the water layer was determined by LSC. The distribution of radioactivity in the water layer was determined by HPLC analysis. After collection, the sediment samples were extracted in three steps: first with 5% v:v acetic acid in methanol, second with methanol:5% acetic acid 50:50 (v:v), and third with 5% (v:v) aqueous acetic acid. The extracts from the three steps were combined. The concentration of radioactivity in the sediment extract was determined by LSC and then the extract was analysed by HPLC.

Findings:

The material balance for the recovery of radioactivity ranged between 103.0% - 96.9%. Most of the radioactivity was detected in the water layer and radioactivity distribution is summarised in Table A1.15 below.

Table A1.15: Mean % AR Recovered in Sample Matrices

Day	Water	Sediment extract	Sediment bound	Organic Volatiles	CO_2	Material Balance
0	101.4	1.6	0.0	NA	NA	103.0
1	96.1	2.3	0.0	0.002	0.015	98.4
3	97.6	2.2	0.0	0.002	0.015	99.8
8	94.2	2.7	0.0	0.002	0.035	96.9
16	96.6	2.8	0.0	0.002	0.086	99.5
23	97.6	3.1	0.1	ND	0.20	101.0
30	95.1	2.5	0.05	ND	0.277	98.0

NA = Not applicable; ND = Not detected.

The percentage AR recovered in the water layer ranged between 101.4% (day 0) and 94.2% (day 8). The radioactivity in the sediment extract did not exceed 3.1%. The mean % AR bound to the sediment was minimal and did not exceed 0.1%. Radioactive CO_2 was the only volatile component determined in the traps, and it increased from 0.015% AR at day 1 to 0.28% AR at day 30, considering both CO_2 traps.

HPLC analysis of the water layer and sediment extract revealed one major component, identified as parent compound, and one minor component. The mean % AR associated with 2,4-D ranged between 103.0% at day 0 and 96.9% at day 8. Because no significant degradation was observed, it was not possible to calculate the half-life. The minor component, was detected only in one replicate at the day 30 sampling interval and accounted for 0.1% AR. Therefore, the entire radioactivity at all other samples found in the water in Table A1.15 above was due to parent compound. Similarly, for sediment extracts, all radioactivity found was due to non-degraded parent compound with the expection of 0.1% AR at day 30.

Conclusion:

Under aerobic aquatic conditions, 2,4-D did not significantly degrade over a 30 day incubation period. In addition to 2,4-D, a small amount of CO_2 (0.28% AR) and one minor component (0.1% AR) were detected by day 30. It appears this test was not conducted for long enough. No results from the preliminary study are available,

although according to the protocol, such a study should have been undertaken in order to determine the amount of degradation and establish intervals to be used in the definitive study. If the preliminary study showed a degree of degradation for the sandy sediment used in this study, it would have been prudent to report this given the lack of degradation found over 30 days. Considering results from the previous two water/sediment tests, it appears likely that degradation may have occurred had the test been carried on as the degradation of 2,4-D has been shown to be biphasic with a much longer initial half-life than the second phase half-life. However, no conclusions to this effect can be drawn from this study.

Water – Anaerobic

Report: Cohen, 1990b; Cohen, 1990c

Guidelines: US-EPA Subdivision N; 162-3

GLP: yes

Test System

The anaerobic aquatic metabolism of 2,4-D, uniformly labelled in the phenyl ring, was studied at a concentration of 4.86 ppm in sieved (2-mm), non-sterilised Louisiana rice paddy sediment (clay soil) and water mixture. The sediment characteristics are described above in Cohen, 1991a.

The redox potential of the sediment/water system was measured before treatment with test substance to assure that anaerobic conditions were established and periodically during the study to assure anaerobic conditions were maintained. Redox determinations were made using an Orion Model 96-78-00 platinum redox electrode and an Orion Model 501 digital ion analyser. Tebbutt (1992) states that anaerobic systems have redox potentials below +50 mV. On day 0, the redox potential was –230 mv and was measured at between –220 and –230 mv for the duration of the study.

Untreated sediment/water mixture was pre-incubated under anaerobic aquatic conditions in darkness at 25.0±0.8^{o}C for 138 days to activate microbes, although the actual level of microbial activity does not appear to be provided in the test report. Following the activation period, ^{14}C-2,4-D in acetonitrile was added to the sediment/water mixture in the incubation flask and mixed thoroughly, resulting in a test system concentration of 4.86 ppm. The test system was then anaerobically incubated in darkness at 25.8±0.8^{o}C for 365 days. The test mixture was continuously purged with humidified nitrogen at 45-50 mL/minute to flush any volatile compounds formed into a series of trapping solutions (ethylene glycol, 1M sulfuric acid, ethanolamine and 5% sodium hydroxide).

The report only states that the water/sediment system weighed 1200 g. The protocol states that 400 g hydrosoil was to be used, so it is assumed this is the case with around 800 mL water used in the system.

Upon application of the test substance, the water/sediment mixture was thoroughly mixed with a spatula for around 15 minutes to ensure homogenous distribution among the system. Samples of the test system were taken immediately after treatment and at 21, 6, 13, 22, 35, 70, 85, 120, 160, 224, 281, 338 and 365 days after treatment. Sediment/water samples were separated by centrifugation and replicate test sediment aliquots were combusted and analysed for total radioactivity by LSC. Additional duplicate aliquots were extracted with 1.5M phosphoric acid mixed with ethyl ether, washed with water, and finally re-extracted with 1N sodium hydroxide solution.

Aliquots of all extracts were analysed by LSC. Duplicate water supernatant samples and trapping solutions were analysed by LSC.

The water supernatant and sediment extracts were analysed by HPLC equipped with both a UV (280 nm) detector and a radioactive flow detector connected in series. Radioactive flow detection allowed for the identification of 2,4-D and its anaerobic aquatic metabolites that were formed at a level ≥0.01 ppm. One dimensional TLC was used as a confirmatory analytical method for the identification of 2,4-D and its anaerobic aquatic metabolites.

Findings

2,4-D metabolised significantly during anaerobic aquatic incubation, with an observed half-life of 41.1 days. The breakdown pattern in water and sediment of both parent and metabolites is shown in Table A1.16 below.

Table A1.16 Proportion of radioactive components(% AR) in water and sediment.

DAT	2,4-D	4-CP	2-CP	Unknowns[1]	Bound	TOTAL
Water						
0	63.0	ND	ND	0.0	-	63.0
1	58.0	ND	ND	3.6	-	61.6
6	59.3	ND	ND	4.1	-	63.4
13	55.7	ND	0.7	4.2	-	60.6
22	52.5	ND	0.5	5.2	-	58.2
35	46.3	2.7	ND	3.2	-	52.2
70	28.9	7.2	ND	2.1	-	38.2
85	18.5	4.1	ND	3.4	-	26.0
120	2.8	4.4	ND	1.2	-	8.4
160	0.8	0.4	ND	2.5	-	3.7
224	ND	ND	ND	2.2	-	2.2
281	ND	ND	ND	1.4	-	1.4
338	ND	ND	ND	1.0	-	1.0
365	ND	ND	ND	1.2	-	1.2
Sediment						
0	29.0	ND	ND	1.8	0.4	31.2
1	28.2	ND	ND	1.8	2.5	32.5
6	29.2	ND	ND	2.7	0.3	32.2
13	26.3	ND	0.4	3.1	0.4	30.2
22	30.6	ND	0.3	3.0	0.4	34.3
35	27.3	5.2	ND	2.7	0.9	36.1
70	14.5	13.4	ND	3.4	3.8	35.1
85	11.5	8.7	ND	3.0	6.7	29.9
120	2.4	5.0	ND	3.7	8.4	19.5
160	0.7	1.5	ND	4.7	8.6	15.5
224	0.3	0.5	ND	2.8	8.2	11.8
281	ND	ND	ND	1.9	5.1	7.0
338	ND	ND	ND	4.5	4.8	9.3
365	ND	ND	ND	2.6	5.9	8.5

1) Unknowns are based on total radioactivity found through LSC less the amount of 2,4-D, 4-CP and 2-CP found through HPLC analysis.
2) ND = not detected

The major metabolite, 4-CP, increased to 20.6% AR by day 70 before declining to 0.5% AR by day 224 and was not detected from then on. A minor metabolite, 2-CP,

reached a maximum of 1.1% AR on day 13 before declining to 0.8% AR by day 22 and was undetected from then on.

The report states CO_2 production reached 71.1% AR by the end of the 365 day study and radioactivity of volatiles was almost exclusively attributed to CO_2. However, this is corrected in the supplementary report where it is indicated that 20.6% AR was attributable to CO_2 at the end of the study.

The mass balances obtained from day 85 onwards were very low (60% or less), and from day 120 until the end of the study were between only 28.5 and 39.9%. These balances are very low. The supplementary report attributes this to an inefficient trapping system for CO_2 (and demonstrates this through argument). The rate of CO_2 production increased relatively rapidly after day 35. The data show that the largest single decline in material balance occurred between days 85 and 120 (20.4%) that correlates exactly with the interval of highest CO_2 production. The author therefore suggests that the material balance deficit was due to the loss of volatile CO_2, particularly since the only volatile compound observed throughout the course of the study was CO_2.

Although the material balance was incomplete, the author argues that the pattern of decline of 2,4-D and the formation and decline of its volatile and non-volatile degradates in the test system was clearly established. Additionally, it is stated the 41.1 d half-life determination yielded a linear regression correlation coefficient (r^2 of 0.9145, indicative of a good fit. It was therefore concluded in the report that the poor mass balance, being due to incomplete trapping of CO_2, in no way affects the half-life of the parent as determined in this study.

The half-life in the report was determined based on log linear regression. DEH has re-plotted the data in Excel, and while the same Log(%) values were derived, different outputs were obtained. DEH derived an r^2 value of 0.9565, a constant of 2.124 and a resulting half-life of 26.6 days. Despite this good correlation, the data still appear to follow a biphasic degradation pattern. The data have been re-analysed by DEH based on this and the following outcomes obtained:

	Phase 1		Phase 2	
	r^2	$t_{1/2}$	r^2	$t_{1/2}$
Water	0.952	52.8	0.976	16.6
Sediment	0.872	64.0	0.926	27.1
Total System	0.939	56.8	0.974	21.5

Conclusion:

Anaerobic degradation of 2,4-D in the water/sediment system described above appears to be biphasic with slower degradation over the first 7-9 weeks (half-life estimated to be around 57 days for the whole system) and significantly faster degradation from then on (half-life around 3 weeks for the whole system).

Report: Concha and Shepler, 1994b

Guidelines: US-EPA Subdivision N; 162-3

GLP: yes

Test System

An anaerobic aquatic metabolism study of ^{14}C-2,4-D, uniformly labelled in the phenyl ring, in viable pond sediment and water from Henry County, Illinois was conducted at an application rate of 5 ppm for 365 days.

Table A1.17 Chemical and physical properties of sediment.

Parameters - Sediment		
Textural Class	Silty Clay Loam	
Sand [%]	2.0	
Silt [%]	66.0	
Clay [%]	32.0	
pH	7.7	
Bulk Density (g/cm^3)	1.17	
Organic Matter (%)	3.11	
WHC (%) @ 1/3 Bar g/g oven dry sediment	41.89	
Cation Exchange Capacity [meqN/100 g dry sediment]	18.58	
	Day 0	Day 35
Redox Potential (mV)	-154	-79
Dissolved Oxygen (ppm)	0.29	16.8
Biomass (CFU/g) Anaerobic Bacteria	2.05X10^6	1.9X10^4
Parameters - Water		
Alkalinity (mg $CaCO_3$/L)	400	
Total Suspended Solids (mg/L)	224	
pH	6.9	
Hardness (mg $CaCO_3$/L)	408	

* following the end of the 281 day activation period.

The test system was maintained at around 25^{o}C and dosed at 4.9 mg/L (equivalent to 19.8 mg/kg sediment). The test systems were prepared by adding 37.1 g moist sediment and 62.8 mL pond water to biometer flasks. This resulted in test systems comprised of 20 g oven dry equivalent of sediment and 80 mL pond water. The dosing solution was delivered to test systems in 1 mL water. A polyurethane foam plug was used for trapping organic volatiles and 10% KOH trapping solution (50 mL) was used for trapping CO_2. Trap solutions and foam plugs were exchanged at weekly intervals and at sampling times. Where redox potentials are less than +50 mV, systems may be considered anaerobic (Tebbutt, 1992). Redox potentials measured at the start and end of this study confirm anaerobicity of the system throughout the test.

Sampling was undertaken at Days 0, 13, 20, 26, 30, 35, 42, 56, 110, 240 and 365. Following separation of the sediment and water, the sediment was extracted three times with acetone:water:acetic acid, 90:5:5 (v:v:v). No extraction was performed on the aqueous samples. LSC was used to determine radiocarbon. HPLC was used to identify the parent and degradates. HPLC and TLC were used for confirmation of degradates. Samples were extracted and radioassayed (LSC) immediately after sampling. Water samples were stored under refrigerated (<10^{o}C) conditions. All other samples and reference standards were stored frozen when not in use.

Findings:

The major component of the aqueous phase was 2,4-D, which represented 25.9% AR 365 days after treatment. In the sediment extracts, parent comprised 13.2% AR at the end of the study giving a total of 39.1% AR in the whole system. Product distribution and metabolite formation patterns are summarised in Table A1.18 below, while the dissipation pattern of 2,4-D and the 2,4-DCP metabolite from water and sediment are summarised in Table A1.19.

Table A1.18: Average Product Distribution and Radiocarbon Recovery (% AR)

Day:	0	13	20	26	30	35	42	56	110	240	365
% 2,4-D	93.3	82.1	76.8	71.0	53.3	68.5	44.0	72.4	68.8	36.1	39.1
% 2,4-DCP	1.0	7.3	11.7	5.9	21.6	15.8	6.9	10.6	17.6	2.2	4.2
% Others*	2.8	2.7	0.8	1.9	2.8	7.6	5.7	3.2	2.1	6.6	5.5
% CO_2	0	0.8	0.7	5.8	0.7	0.5	16.2	1.7	0.8	11.9	22.1
% Organic volatiles	0	0.4	0.1	0.3	1.0	0.5	0.7	0.5	2.0	2.2	2.1
% Bound	2.3	8.5	8.8	16.7	21.1	21.3	28.7	14.4	8.5	34.7	26.5
TOTAL	99.4	101.8	98.9	101.6	100.5	114.2	102.2	102.8	99.8	93.7	99.5

* This category is comprised of several zones by HPLC with no observed discernible pattern of rise and decline.

Table A1.19: 2,4-D residues (% AR) dissipation from water and sediment:

	2,4-D			2,4-DCP		
DAT	**Water**	**Sediment**	**Total**	**Water**	**Sediment**	**Total**
0	79.7	15.5	95.1	0.6	0.4	1.0
13	68.1	14.0	82.1	2.7	4.6	7.3
20	51.4	25.4	76.8	4.8	7.0	11.8
26	56.2	14.9	71.0	1.7	4.3	5.9
30	42.0	11.3	53.3	7.7	13.9	21.6
35	45.6	23.1	68.6	5.0	10.8	15.8
42	30.5	13.6	44.1	2.2	4.8	7.0
56	61.0	11.4	72.4	4.2	6.5	10.7
110	58.1	10.8	68.8	7.4	10.3	17.6
240	23.7	12.4	36.1	0.8	1.4	2.2
365	25.9	13.2	39.1	1.3	2.9	4.2

The two major metabolites observed were 2,4-DCP, which rose to 21.6% AR at day 30 and subsequently declined to 4.2% AR after 365 days of exposure, and CO_2. Small unidentified peaks with random retention times were detected in some HPLC radiochromatograms. However, no pattern of formation/decline for any of these peaks was apparent during the study. The largest unknown at 365 days represented 1.5% AR. In addition, small quantities of volatiles were extracted from the foam plugs. At 365 days these included 4-chlorophenol (1.9%), 2,4-DCA (0.7%) and 2,4-DCP (0.7%).

Linear regression of the log-transformed data describing 2,4-D degradation with time resulted in an approximate half-life of 312 days ($r^2 = 0.69$) for anaerobic aquatic metabolism.

The half-life calculated by the authors above used only the replicate B from the day 42 sample. The r^2 value is still not particularly good. DEH has replotted the data. Considering all data available based on means of two replicates, the r^2 value for the linear regression equation obtained is 0.5688 and the calculated half-life is 334 days. To try and improve the correlation of the regression equation, apparent outlying

samples can be removed. For example, considering the data in Table A1.19 above, the total radioactivity observed for 2,4-D on days 30, 42 and 240 appear significantly lower than would be expected based on the other data. If these are removed from the equation, the r^2 value is significantly improved (0.9031). Interestingly though, the predicted half-life remains the same at 334 days. The half-life for the water compartment, worked out in the same manner, is predicted to be around 273 days (r^2 = 0.76).

Conclusion:

2,4-D is expected to be persistent in anaerobic aquatic situations with a half-life >250 days in water and >300 days for the whole system predicted on the above study.

Mobility

Adsorption/Desorption

Report: Fathulla, 1996b

Guidelines: US-EPA Subdivision N; 163-1

GLP: yes

Test System

The adsorption and desorption characteristics of 2,4-D, uniformly labelled in the phenyl ring, was investigated on four representative agricultural soils with the following characteristics:

Table A1.20: Soil characteristics

Texture	Origin	% Sand	% Silt	% Clay	OC[1]/OM	CEC (meq/100 g)	pH	FC[2]
Sand	Plainfield	91	5	4	0.47/0.8	3.5	5.6	3.1
Sandy Loam	California	65	27	8	0.59/1.0	5.7	6.7	9.6
Loam	Mississippi	44	48	8	0.24/0.4	9.3	7.0	11.9
Silty Clay Loam	Airzona	12	58	30	0.88/1.5	44.3	7.9	33.1

1) % Organic Carbon = % Organic Matter/1.72
2) g H_2O/100 g dry soil.

Range finding investigations were conducted on 2,4-D to determine the potential adsorption to glass, volatility, stability under adsorption test conditions, adsorption equilibrium time and proper soil:solution ratio. The results showed the following: 2,4-D did not adsorb to glass, nor did it volatilise, 2,4-D was stable under adsorption test conditions and proper soil:solution ratio was 1:1.

For the adsorption/desorption experiment, samples were prepared at four nominal concentrations of 1, 2.5, 5 and 10 ppm in aqueous 0.01M calcium chloride for each soil. The samples were equilibrated in a shaking water bath for 24 hours at around 25^{o}C followed by vortexing and centrifugation. The resulting supernatant was analysed by LSC to determine the disappearance of radioactivity from solution as a measure of test material adsorption to soil. The supernatant was then removed from each sample and replaced with an equal volume of untreated 0.01M $CaCl_2$. The samples were equilibrated for a further 24 h followed by vortexing and centrifugation. The supernatant was analysed by LSC to determine desorption. The radioactivity remaining in the soil after desorption was determined by oxidation followed by LSC analysis.

Findings

Mass Balance

The material balance results for the definitive study for all four soils ranged from 96.4-107.3%

Transformation of Parent Compound

Hours 0 and 24 stability samples were analysed by HPLC. Between 94.7% and 108.7% of the applied radioactivity initially present was detected in the adsorption supernatants and soil extracts as parent compound indicating 2,4-D was stable in the test system over 24 hours.

Additionally, the high dose samples desorption supernatants from the four soil types were analysed using HPLC for stability determination after 48 hours. The results showed the test material accounted for 100% radioactivity in the desorption solution.

Results

A general pattern of increased percentage of 2,4-D adsorption to soil with decreasing concentration was observed for all soil types. Regression analysis of the solution and soil log concentration at all treatment levels was linear in all soil types indicating that adsorption to soil followed the Freundlich equation. Correlation coefficients for adsorption were above 0.99 for all soils except the California sandy loam where it was 0.821.

Table A1.21: Freundlich Adsorption and Desorption Coefficients for 2,4-D

	Adsorption			Desorption		
Soil	% ads	K_d	K_{oc}	% des	K_d	K_{oc}
Sand	20.2-27.8	0.357	76	48.2-51.4	1.16	247
Sandy loam	8.1-17.7	0.167	70	39.1-55.6	0.811	338
Loam	14.9-22.0	0.281	117	38.3-51.3	1.48	617
Silty clay loam	27.3-37.6	0.517	59	36.2-43.8	1.90	216

Using linear regression, a weak correlation was found between OM and pH to K_d values (r^2 of 0.584 and 0.430 respectively) while much better correlations were found between clay contents and cation exchange capacity and K_d values (r^2 of 0.768 and 0.823 respectively).

Conclusion:

Interpreting these results using the adsorption Koc, and based on the McCall Mobility Class scale (McCall *et al*, 1980), the chemical can be classed as having high mobility (Koc 50-150) in all four soils. Clay content and cation exchange capacity appear to be the main factors impacting mobility.

Report: Cohen, 1991b

Guidelines: US-EPA Subdivision N; 163-1

GLP: yes

Test System

The adsorption and desorption characteristics of 2,4-D, uniformly labelled in the phenyl ring, was investigated in non-sterilised Louisiana rice paddy sediment. Soil characteristic are reported above in Cohen, 1991a.

Two scoping studies were conducted to determine the adsorption equilibrium time (24 hours) and the desorption equilibrium time (8 hours). It was determined through the scoping studies that 2,4-D does not bind to the glass surface.

For the adsorption/desorption experiment, samples were prepared at five concentrations of 0.1, 0.51, 1.0, 2.47 and 5.02 ppm in 0.01M calcium chloride. Duplicate aliquots were assayed by LSC to determine the exact concentration. 15 mL of each treatment were added to 5 g sieved (2-mm) sediment in tared 40 mL glass centrifuge tubes with PTFE-lined screw caps. Each concentration of treatment solution was set up in duplicate and mixed thoroughly by vortexing for 2 minutes. The treated mixtures were then incubated in the dark at around 22^{o}C for the 24 h equilibration period.

Following this, test samples were centrifuged. The supernatants were decanted and duplicate aliquots (0.1 mL) of each aqueous fraction analysed by LSC. An additional aliquot of solution was analysed by HPLC and the weights of the adsorbed sediments were determined.

For desorption, the sediments were mixed with an equal volume (15 mL) of freshly prepared calcium chloride at around 22^{o}C. The tubes were resuspended by vortexing for 2 minutes then rotated for 8 hours and centrifuged. Following this, the aqueous solutions were decanted. The weight of the desorbed sediment was determined and analysed by combustion followed by LSC. Duplicate aliquots of each desorption supernatant were analysed by LSC. An additional aliquot of supernatant was analysed by HPLC.

Findings

Mass Balance

The average material balance for the five concentrations was 98.9% (range of 94.8-101.3%)

Transformation of Parent Compound

The study report does not discuss the HPLC analysis of samples to consider the potential breakdown of 2,4-D. However, the HPLC chromatograms for the highest concentration (5.02 ppm) are provided for time 0 and 2 replicates at the end of the 8 h desorption phase. The peak in the time 0 chromatogram indicates around 94% 2,4-D while the average area for the 2,4-D peaks in the two chromatograms following desorption is also around 94%. This indicates that there was negligible transformation of the parent compound during the study.

Results

The $K_{d\text{-}ads}$ value for 2,4-D was 1.22. This is indicative of a significant amount of chemical being adsorbed to sediment from water in the 24 hours equilibration. However, the K_{oc} value of 58.1 suggests the material is not tightly bound. This is supported by the $K_{d\text{-}des}$ value of 1.64 (corresponding $K_{oc\text{-}des}$ of 78.1). Values for 1/n were 0.83 and 0.74 for the adsorption and desorption curves respectively.

Conclusion:

Interpreting these results using the adsorption Koc, and based on the McCall Mobility Class scale (McCall *et al*, 1980), the chemical can be classed as having high mobility (Koc 50-150) in the tested sediment.

Report: McCoy and Lehman, 1988

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

GLP: Yes

Test System

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

Table A1.22: Soil characteristics

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

Prior to the adsorption study, a preliminary study was conducted to determine the time needed for the soil/water slurry to achieve equilibrium. Four g of Mahoon soil were shaken in duplicate with 10 mL of 5 ppm 2,4-D solution in the dark with tubes removed at various times up to 24 h. Following centrifugation, aliquots were analysed by LSC. The samples were then resuspended and placed back on the shaker until the next sampling time. A plot of counts indicated that equilibration had been reached by 21 hours, therefore, 24 hours was chosen for the equilibration time in the main study.

For the definitive study, a solution containing 2,4-D was equilibrated in these soils at four different initial concentrations (0.2, 1.0, 2.5 and 5.0 ppm). A soil:water ratio of 1:2.5 was used. For each soil, 4 g were shaken in duplicate with 10 mL of the various 2,4-D solutions in the dark for 24 h. A control with no soil was prepared and treated in the same manner.

After equilibration, the tubes were centrifuged and aliquots removed for analysis by LSC. A further aliquot of supernatant from the 5 ppm sample was analysed by HPLC. The remaining soil and the control tube were extracted with 10 mL of a solution containing 94% methanol, 5% water and 1% 1N HCl. Following extraction, the tubes were centrifuged and the extract sampled. The determine the remaining ^{14}C activity, the dried soil was combusted.

Findings:

Mass balance:

The recovery for the four soils at all 2,4-D concentrations ranged from 93.3% to 108.5% with an average recovery of 100.2%.

Parent Transformation:

HPLC analysis of the supernatant from each soil for the 5 ppm treatment level showed the 2,4-D peak accounted for 98.9-99.7% of the radioactivity in the samples indicating there is no apparent degradation of the parent compound.

Results:

Following adsorption, the majority of the radioactivity was found in the water supernatant with generally between 70-80% AR in all soils at all concentrations

except the Hanford sandy loam soil where almost all AR was in the supernatant. Of the radioactivity remaining in the soil, slightly more was extractable than bound with generally around 14% extracted from the silt loam, loam and clay and generally around 10% remaining bound in these soils. Only 3-4% AR remained with the soil in the Hanford sandy loam soil, and of this, < 1% AR remained bound with the soil following extraction at all concentrations.

The following Koc values were obtained:

Table A1.23: % OC, K_d and K_{oc} adsorption values.

	Adsorption		
Soil	% OC	K_d	K_{oc}
Sand	2.23	0.94	42
Sandy loam	0.22	0.08	34
Loam	3.08	1.1	36
Silty clay loam	1.26	1.0	79

Conclusion:

Interpreting these results using the adsorption Koc, and based on the McCall Mobility Class scale (McCall *et al*, 1980), the chemical can be classed as having high mobility (K_{oc} 50-150) in the silty clay loam and very high mobility (K_{oc} 0-50) in the other three soils.

Leaching Potential

Column Leaching Studies

No column leaching data were provided for unaged 2,4-D residues. One study of this sort is described in the EU report and this is being sought. It is a gap in the data as aged residue leaching studies described below meant very little parent compound remained in the system prior to leaching. Soil metabolism data indicated 2,4-D dissipates in a biphasic nature with a rapid half-life after the first 20-30 days. The ageing period appeared sufficiently long for this second phase to take effect, thereby resulting in significant removal of 2,4-D and significant production of CO_2. Leaching studies for the unaged parent compound are considered important as the chemical has been predicted to be mobile in batch equilibrium studies described above, and is persistent in anaerobic systems, therefore, more likely to be persistent in groundwater.

Aged Column Leaching Studies

Report: Zohner, 1990a

Guidelines: US-EPA Subdivision N; 163-1

GLP: Yes

Test System

Leaching characteristics of aged residues of ^{14}C-2,4-D uniformly labelled in the phenyl ring were determined in a sand soil with mild humus content. The characteristics of the soil were as follows:

Texture	Series	% Sand	% Silt	% Clay	OC	CEC (meq/100 g)	pH	FC[1]
Loamy sand	Speyer 2,1	82.9	11.7	5.4	0.6	6.8	7.3	27

1) g H_2O/100 g dry soil.

The soil was stored outdoors under natural moisture prior to use. For the experiment, it was sieved (2-mm) and biological activity determined. Microbial activity was low and the soil pH (5.4 before amendment) was not in the recommended pH range. Therefore, the soil was amended with 0.5% alfalfa meal and 1 g/kg calcium carbonate and preincubated for 1 week prior to treatment with 2,4-D.

For soil treatment and ageing, a radioactive treatment solution of 2,4-D was prepared in aqueous phosphate buffer at pH 7. Soils were dried slightly to around 20% maximum water capacity to facilitate homogenizing. The soil was treated with the stock solution under stirring and the theoretical test concentration was 1.4 mg/kg (around 1 kg/ha when mixed in the top 5 cm soil of density 1.5 g/cm^3). After application of stock solution, the soil was readjusted to 40% maximum water holding capacity (around 75% field capacity). Thereafter, the treated soil was aerobically incubated for 28 days at around 20^{o}C in the dark in metabolism flasks. Aerobic conditions were achieved by continuous aeration. Carbon dioxide in the purging air was trapped in 2 KOH traps.

For the leaching part of the experiment, air-dried and sieved soil was packed up to 28 cm in a glass column. This was saturated with 0.01 N calcium chloride solution until leachate could be collected. 150 g of the aged soil was placed in a layer of 5.3 cm on the top of the soil column. Percolation was done with 1121 mL 0.01N calcium chloride solution at a rate that maintained a constant head of water of 10 cm above the soil surface. Precautions were taken to minimise evaporation the leachate. The leaching period took around 65 hours after which the soil was removed from the glass column and divided into 6 segments of around 5 cm length each.

Leachate was collected in fractions and measured for radioactivity. The leached radioactivity was analysed by TLC. The sections of soil columns were analysed for extractable and non extractable radioactivity. Soils were extracted in a soxhlet apparatus with acetone for 1.5 hours and then with the same volume of water saturated n-butanol for an additional 4.5 hours. The extracts were measured for total radioactivity by LSC, pooled, concentrated by evaporation and characterised by TLC. Extracted soil samples were air dried and homogenised. Radioactivity was determined by oxidation followed by LSC of trapped $^{14}CO_2$. A material balance was calculated with the recovered radioactivity from the soil and leachate.

Findings:

Mass Balance

At day 0, 98.11% AR was recovered. After the 28 day ageing period, 89.05% AR was collected and consisted of 5.39% AR extractable, 14.51% non-extractable and 69.15% as CO_2. The incomplete recovery of radioactivity after this time was considered due to incomplete recovery of $^{14}CO_2$ from soil by purging the soil with air during the ageing period. Remaining radioactive carbon dioxide in the soil was volatilised during the hot soxhlet extraction. It was not recovered therefore in the organic extract and got lost from the material balance. This theory was confirmed to some extent by the detection of some $^{14}CO_2$ in the leachate.

Transformation of Parent

TLC analysis was performed on the top soil segment only as radioactivity in the lower soil segments was too low for analysis. The greatest portion of the radioactivity remained on the origin of the chromatograms and represented various not resolved highly polar degradates. 38.2% of the radioactivity extractable after 28 days (2.06%

AR) volatilised during the concentration by evaporation process. Of the initially applied radioactivity, only around 3.3% AR remained available for leaching (5.39% AR extracted with 38.2% of this volatilising). TLC analysis of this residue revealed around 2.5% AR being attributed to the polar degradates mentioned above, and 0.8% AR being parent compound.

Results

Following leaching with 1121 mL 0.01N calcium chloride solution, a total of 1121 mL effluent was collected in six fractions. 0.27% AR (equivalent to 0.0005 mg/L of 2,4-D equivalents) in the pooled leachate was found. Depending on the concentration method, 12.7-20.6% of this was 2,4-D amounting to around 0.0001 mg/L in the pooled leachate. The metabolite 2,4-DCP was not detectable on chromatograms.

Conclusion:

Under the conditions of this test, 2,4-D is not expected to leach to groundwater when residues are aged. The loss of 2,4-D during the ageing process along with corresponding high production of CO_2 is consistent with aerobic soil metabolism data described above.

Report:	Zohner, 1990b
Guidelines:	BBA-Guidelines Teil IV, 4-2
GLP:	Yes

Test System

The leaching characteristics of aged residues of ^{14}C-2,4-D uniformly labelled in the phenyl ring were determined in largely the same test system as described above in Zohner 1990a to conform with BBA guidelines. The soil was the same as that used in Zohner 1990a above and amended in the same manner to increase microbial activity and pH.

In this test the theoretical test concentration was 2.19 mg/kg (around 1 kg/ha when mixed in the top 3 cm soil of density 1.5 g/cm^3) and the period of ageing was for 30 days.

In the leaching part of the experiment, air-dried and sieved soil was packed up to 28 cm in a glass column. This was saturated with water until leachate could be collected. 112.1 g of the aged soil was placed in a layer on the top of the soil column. Percolation was done with 441 mL of water at a rate that maintained a constant head of water of 10 cm above the soil surface. Precautions were taken to minimise evaporation of the leachate. The leaching period took around 27 hours after which around 431 mL of leachate had been collected. The leachate was used for total radioactivity determinations by LSC and the percolate fractions pooled and submissted to TLC analysis.

Leachate was collected in fractions and measured for radioactivity. The leached radioactivity was analysed by TLC. There was no sectioning of soil columns in this study.

Day 0 and Day 30 soil samples were extracted as described in Zohner 1990a. The extracts were measured for total radioactivity by LSC, pooled, concentrated by evaporation and characterised by TLC. Extracted soil samples were air dried and

homogenised. Radioactivity was determined by oxidation followed by LSC of trapped $^{14}CO_2$.

Findings:

Mass Balance

At day 0, 98.11% AR was recovered. After the 30 day ageing period, 87.84% AR was collected and consisted of 4.08% AR extractable, 12.19% non-extractable and 71.57% as CO_2. The incomplete recovery of radioactivity after this time was considered due to incomplete recovery of $^{14}CO_2$ from soil by purging the soil with air during the ageing period. Remaining radioactive carbon dioxide in the soil was volatilised during the hot soxhlet extraction. It was not recovered therefore in the organic extract and got lost from the material balance. This theory was confirmed to some extent by the detection of some $^{14}CO_2$ in the leachate.

Transformation of Parent

Following ageing, the radioactivity of the pooled soil extracts was too low for direct TLC measurement. The greatest portion of the radioactivity remained on the origin of the chromatograms and represented various not resolved highly polar degradates. Of the initially applied radioactivity, only around 4.1% AR remained available for leaching. TLC analysis of this residue revealed around 3.82% AR being attributed to the polar degradates mentioned above, and 0.23% AR being parent compound.

Results

Following leaching with 441 mL, a total of 431 mL effluent (average from two columns) was collected in nine fractions. 0.34% AR (equivalent to 0.0018 mg/L of 2,4-D equivalents) in the pooled leachate was found. In terms of % AR submitted to ageing, 0.007% (average of two columns) of 2,4-D was found in the leachate. This is a concentration of <0.04 ppb. The metabolite 2,4-DCP was not determinable on chromatograms.

Conclusion:

Under the conditions of this test, 2,4-D is not expected to leach to groundwater when residues are aged. The loss of 2,4-D during the ageing process along with corresponding high production of CO_2 is consistent with aerobic soil metabolism data described above.

Lysimeter/Field Leaching Studies

No studies provided.

Fate and Behaviour in Air

The calculated Henry's Law Constants of 3.54×10^{-8} atm.m^3/mol (calculated from VP/Sol) is indicative of very slight volatility from water (Mensink *et al*, 1995).

No experimental data for degradation or volatility in the atmosphere were provided. However, the former was considered through modelling.

The rate constant for reactions of 2,4-D 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. The structure of 2,4-D acid was entered into the program with the following SMILES notation: c1cc(Cl)cc(Cl)c1(OCC(=O)O).

First, the rate constant k_{OH} of the active substance was estimated based on the chemical structure. The resulting value was

$$6.6262 \text{ X } E^{-12} \text{ cm}^3\text{/molecule.sec}$$

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

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

The diurnally and seasonally averaged concentration of tropospheric hydroxyl radicals used by the AOP program is $1.5 \text{ X } 10^6$ cm^{-3}. Therefore, half-life for the degradation of 2,4-D by hydroxyl radicals was calculated to be 1.61 days based on a 12 h:12 h light:dark day.

Accumulation/Bioaccumulation

Given the properties of 2,4-D (high water solubility and low octanol-water partition co-efficient), it would not be expected to accumulate in biota to any extent. Wang *et al*, 1994 considered the accumulation of 2,4-D in fish and water hyacinth. ^{14}C-2,4-D was labeled at the 2-position of acetic acid for use in the test. Two fish, Carp (*Cyprinus carpio*), 3.5-4.0 cm long and tilapia (*Orechromis mossambicus*), 3.0-3.5 cm long were used as test fish. Water hyacinth (*Eichhornia crassipes*), 30 g each plant, was collected from a ditch in Hsin-chu, Taiwan.

Exposure rates for the fish were 0.5 and 0.05 ppm. The weight of all fish in a test did not exceed 1 g/L of water. Tests were conducted in duplicate and experiments performed without aeration. Absorption and accumulation of 2,4-D by water hyacinth was performed by cultivating 10 well-grown plants in a plastic tank containing 10 L of water amended with 0.01 or 0.001 ppm. Experiments were performed out-of-doors and liquid quantities kept constant by adding distilled water daily.

At designed intervals (0, 0.5, 1, 2, 3, 5, 7 and 14 days), fish or plants were removed and washed with clean water prior to extraction and analysis. Recovery of 2,4-D from fish was 93.7% while from the leaf/stem and root from water hyacinth was 86.6 and 72.7% respectively.

Round 83% (0.5 ppm group) and 91% (0.05 ppm group) of the radioactive matter remained in the water until 14 days after the start of the test. Generally, a maximum accumulation of radioactivity in fish was achieved 5-7 days after commencement of exposure. No significant variance was shown in the accumulation of the concentration in fish. The higher the initial concentration in water, the more accumulation in fish was observed. Nonetheless, BCF values (calculated as radioactivity in water/radioactivity in fish) ranged between 11.8 (0.5 days in carp) to 45.4 (7 days in carp) for both fish species in the 0.5 ppm exposure group, and 6.2 (0.5 days in carp) to 26.7 (5 days in carp) in the 0.05 ppm exposure group. These low values support the theory that 2,4-D is unlikely to bioconcentrate or bioaccumulate.

In the plant test, herbicide was absorbed by water hyacinth, and a part of the chemical transported to leaf and stem from the root. The authors report that uptake in the 0.01 ppm group was around 10 times more than that in the 0.001 ppm group (although the data as reported in the paper are not clear on this). BCF values were not calculated. However, where BCF is taken to be residues in water/residues in plant, without correcting for recoveries, the BCF in the 0.01 ppm group was shown to be between 7.9-14.3, again indicating a low potential to accumulate.

Avian Toxicity

Acute

Two non-standard oral avian toxicity studies were provided to the APVMA. Results are summarised as follows:

Table A1.24. Summary of Acute Bird Toxicity Results for 2,4-D Acid

Test substance	Species	LD50 (mg/kg bw)	Reference
2,4-D Acid[1]	Domestic chicken	1064	Chittibabu, 2002a
2,4-D Acid[1]	Pigeon	999	Chittibabu, 2002b

1) Non-Standard test.

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

Report: Chittibabu, 2002a

Guidelines: Gaitonde committee Guideline (6.4.0.Di)

GLP: No (Quality Assurance Statement provided)

Test System

The acute oral toxicity of 2,4-D Acid technical 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 vegetable oil to obtain a homogenous test solution. All birds were starved overnight prior to dosing by oral intubation. Dose rates were 500, 950 and 1805 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, 500, 950 and 1805 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 950 mg/kg bw group occurred on day 7, while in the highest treatment group, 1 bird died on day 6, 9 and 14. In this highest treatment group, birds exhibited dullness and incoordination after 72 hours, while birds treated with 950 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 1064.4 mg/kg bw with the confidence limits (assumed to be 95%) ranging from 737.1-1392.1 mg/kg bw.

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

Test System

The acute oral toxicity of 2,4-D Acid technical 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 vegetable oil to obtain a homogenous test solution. All birds were starved overnight prior to oral intubation. Dose rates were 500, 900 and 1620 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, 500, 900 and 1620 mg/kg bw groups was 0, 0, 1 and 3 birds respectively corresponding to 0, 0, 33.3 and 100% respective mortality. The only death in the 950 mg/kg bw group occurred on day 6, while in the highest treatment group, 2 birds died on day 5 and the remaining bird on day 6. In this highest treatment group, birds exhibited dullness and incoordination after 48 hours, while birds treated with 900 mg/kg bw exhibited dullness alone. Birds in the lowest treatment group and control did not exhibit any signs of toxicity.

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

Conclusions:

The acute oral LD50 of 2,4-D technical to the pigeon was calculated to be 998.9 mg/kg bw with the confidence limits (assumed to be 95%) ranging from 717.6-1280.3 mg/kg bw.

In addition, the US EPA report contains one test result as follows:

Species	LD50 (mg ai/kg)	MRID No.
Mallard duck *(Anas platyrhynchos)*	>5620	415462-02

No test was provided to the US EPA for the bobwhite quail.

Short-Term

Three tests were provided to the APMVA for this endpoint with results as follows:

Table A1.25. Summary of Short-Term Bird Toxicity Results for 2,4-D Acid

Test substance	Species	LD50 (mg/kg diet)	NOEC (mg/kg diet)	Reference
2,4-D Acid	Mallard duck	>5620	1000	Culotta *et al*, 1990a
2,4-D Acid	Bobwhite quail	>5620	1000	Culotta *et al*, 1990b
2,4-D Acid	Japanese quail	>5000	5000	Chittibabu, 2002i

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

Report: Culotta *et al*, 1990a

Guidelines: FIFRA Guideline 71-2

GLP: yes

Test System

The study aimed to evaluate the toxicity of 2,4-D when administered to juvenile mallard ducks (*Anas platyrhynchos*) in the diet for 5 days. Nominal test levels were 562, 1000, 1780, 3160 and 5620 ppm ac. The test consisted of an acclimation period of 9 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 four 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. An amount of food sufficient to last the 5 day exposure period was presented to the birds at the initiation of the test with a study of the stability of the test substance in avian diet conducted prior to test initiation. During the test, the average temperature in the brooding compartment of the pens was around 32^{o}C, average ambient room temperature was around 23^{o}C and the average relative humidity was around 67%. 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 study at all concentrations except one bird in the 5620 ppm group that was noted as having a reduced reaction to external stimuli, a ruffled appearance and lethargy on Days 3 and 4. This bird seemed normal for the remainder of the study.

In the four control groups, body weight gains ranged from an average of 120-144 g over the 5 day exposure period. The body weight gains in the 562 and 1000 ppm groups were comparable to this (119 and 124 g average respectively). However, there was a reduction on the body weight gains in the 1780 and 3160 ppm groups compared to the controls, with average gains of 92 and 24 g respectively. In the highest test group, body weights actually dropped by an average 14 g over the 5 day exposure period. A corresponding reduction in feed consumption was noted in the 3160 and 5620 ppm groups. Following exposure, birds in the control group increased in body weight by an average of 92-106 g (days 6-8). Higher increases were observed in the 3160 and 5620 ppm groups over this period of an average of 118 and 123 g respectively.

Conclusion

The dietary LC50 value for mallards exposed to 2,4-D in the diet is greater than 5620 ppm. The NOEC and LOEC from these results are 1000 ppm and 1780 ppm respectively.

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

Test System

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

The birds were 10 days of age at the initiation of the study. Birds were assigned to 5 test groups and four 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. An amount of food sufficient to last the 5 day exposure period was presented to the birds at the initiation of the test with a study of the stability of the test substance in avian diet conducted prior to test initiation. During the test, the average temperature in the brooding compartment of the pens was around 40^{o}C, average ambient room temperature was around 24^{o}C and the average relative humidity was around 25%. 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

In the 1780 ppm group, one mortality was observed on day 2 and 2 further mortalities on day 4. All three birds had lesions of picking. None of the birds exhibited signs of treatment related toxicity. No mortalities were found in the higher exposure group of 3160 ppm, supporting the conclusion that these deaths were not treatment related.

In the 5620 ppm group, one mortality was recorded on day 4, two on day 5 and one on day 6. In this group, signs of toxicity were first observed on the morning of day 4. These signs included lethargy, reduced reaction to external stimuli, ruffled appearance and wing droop. From the morning of day 6 until study termination, all remaining birds in this group were normal in appearance and behaviour.

In the four control groups, body weight gains ranged from an average of 10-12 g over the 5 day exposure period. The body weight gains in the 562 and 1000 ppm groups were comparable to this (10 and 11 g average respectively). However, there was a reduction on the body weight gains in the 1780 and 3160 ppm groups compared to the controls with average gains of 6 and 7 g respectively. In the highest test group, body weights actually dropped by an average 1 g over the 5 day exposure period. There was no apparent treatment related decrease in feed consumption (despite lower body weight gains in the higher three exposure groups) over the exposure period of the study. However, average feed consumption in the 5620 ppm group over the post exposure observation period was only 9 g average per bird compared to 14-30 g per bird in all other treatment and control groups.

Conclusion

The dietary LC50 value for bobwhite quail exposed to 2,4-D in the diet is greater than 5620 ppm. The NOEC and LOEC from these results are 1000 ppm and 1780 ppm respectively.

Test Substance:	**2,4-D**
Report:	Chittibabu, 2002i
Guidelines:	OECD Guideline 205
GLP:	Yes

Test System

The dietary toxicity of 2,4-D Acid technical was assessed on the Japanese quail (*Coturnix japonica*). Ten birds per pen of 3 groups were tested with birds acclimatised for 7 days prior to exposure. Birds were housed in pens with controlled temperature and humidity and provided with a 12 h light:dark photoperiod. Based on a pilot study, the test was conducted as a limit test. Two groups were used as control groups and offered basal diet mixed with carrier alone. The treatment group was exposed to 5000 ppm in the diet for five consecutive days. All birds had an access to water *ad libitum*.

All birds were observed daily for 8 days (assumed to include a 3 day post exposure period, but not reported). Daily food consumption was measured. Body weight of each bird was recorded just prior to the start of the experiment and on days 5 and 8. All birds were observed for toxicity signs twice on day 1 and once daily for the rest of the test. Mortality observations were made daily. All birds were necropsied for gross pathological observations on day 9.

Changes in body weight gain by the birds belonging in the experimental group were compared with that of the control birds using Student's t-test.

Findings:

No mortality was observed in the treated group or either of the control groups. No clinical signs of toxicity related to treatment were observed.

Body weight gain by the test substance treatment group was similar to that of the control groups of birds. Food consumption of the groups of birds (both treated and control groups) was similar. However, there were some changes in the daily feed consumption, which were considered to be incidental as the changes were not uniform throughout the observation days.

Gross pathology conducted at termination revealed petechiae in lungs. Only one bird from each group showed this lesion and was considered to be incidental and not a test substance related specific target organ effect.

Conclusion:

The dietary LC50 from exposure of Japanese quail to 2,4-D acid in the diet was >5000 ppm.

Reproduction

Test Substance:	**2,4-D**
Report:	Mitchell *et al* 2000
Guidelines:	FIFRA Guideline 71-4/OECD Guideline 206
GLP:	yes

Test System

The effects of dietary exposure of 2,4-D on adult Bobwhite quail (*Colinus virginianus*) was evaluated over a period of approximately 21 weeks. 64 males and 64 females were randomly distributed into 1 control and 3 treatment groups. Study phases consisted of acclimation (7 weeks); pre-photostimulation (7 weeks); pre-egg laying with photostimulation (4 weeks); egg laying (approx. 10 weeks); and post adult termination (final incubation, hatching and 14 day offspring rearing period – 6 weeks).

Each group contained 16 pairs of 1 male and 1 female per pen with exposure concentrations in the diet of 0 (control), 160, 400 and 1000 ppm. Homogeneity of the test substance in the diet was evaluated through samples collected (top, middle and bottom of the left and right sections of the mixing vessel) on day 0 of week 1, then with additional samples collected during weeks 2, 3, 4, 8, 12, 16 and 20 of the test to measure/verify test concentrations.

The birds were 21 weeks of age at test initiation and ranged from 180-239 grams in weight. All adult birds were observed daily throughout the test for signs of toxicity or abnormal behaviour. Adult body weights were measured at test initiation, on weeks 2, 4, 6, 8 and at adult termination and feed consumption was measured weekly during the test. At the beginning of week 8, the photoperiod was increased to induce egg production. Following the start of egg production, eggs were set weekly for incubation. Indiscriminate egg samples were taken for determining egg shell thickness and remaining eggs were candled prior to incubation to detect cracks or abnormalities. Eggs were also candled twice during incubation to detect infertile eggs or embryo mortality.

On day 21 of incubation, eggs were moved to an incubator for hatching. Following hatching, hatchlings were removed and group body weight determined. At 14 days of age, the average body weight by parental pen of all surviving offspring was determined. Following test completion, statistical analyses were performed to determine differences between groups.

Findings:

The results of the diet analysis demonstrated that nominal concentrations were acceptable for reporting with mean measured concentrations of 147, 382 and 962 ppm in the test diets.

Biological results: There were no treatment related mortalities, overt signs of toxicity or treatment related effects on body weight or feed consumption at any of the test concentrations. Two deaths were recorded in the 1000 ppm treatment group, one through a bird being inadvertently killed during body weight determination and the other through apparent physical injury. Observed values for the various reproductive parameters are provided below.

Table A1.26: Summary of Reproductive Performance from a Bobwhite Quail Reproduction Study with 2,4-D, Normalised as percentages[1] unless otherwise indicated.

	Experimental Group (mg/kg)			
Reproductive Parameter	**Control**	**160**	**400**	**1000**
Number of Replicates	16	16	16	16
Eggs laid (% of maximum laid)	75.2	79.4	77.4	73.5
Eggs Cracked/eggs laid (%)	1.3	1.9	1.6	2.7
Viable Embryos/eggs set (%)	91	78	96	91
Live 3-Week Embryos/viable embryos (%)	99	98	99	99
Hatchlings/Live 3 week embryos (%)	95	91	92	94
14-Day Old Survivors/Hatchlings (%)	94	96	95	93
Hatchlings/eggs set (%)	85	68*	86	86
14-d old survivors/eggs set (%)	80	68*	82	80
Hatchlings/Maximum set	64	55	67	60
Mean Egg Shell Thickness (mm)[2]	0.226	0.228	0.239	0.232
Mean Hatchling Body Weight (g)[2]	6	6	6	6
Mean 14-Day Body Weight (g)[2]	26	26	26	26

1) Values represent pen means for each experimental group.
2) Represents the absolute mean value (not percentages) in each group.

The reduction of viable embryos as a percentage of eggs set noted in the 160 ppm treatment group were primarily the result of data from one pen from which none of the eggs laid were fertile. The male was noted with quiescent testes at necropsy. This had an impact on the number of hatchlings and the number of 14-day old survivors in

terms of percentage of eggs set, both of which were deemed statistically significantly different from the control. When the data from this pen were removed from calculations, the mean values were no longer statistically significant and were comparable to the control mean values. This is not considered to be treatment related, a theory supported by the non-significantly different results found at higher treatment levels.

Conclusions:

No treatment-related mortalities, overt signs of toxicity or treatment-related effects upon body weight or feed consumption were found at any of the concentrations tested. Additionally, there were no treatment related effects on any of the reproductive parameters measured. The NOEC for northern bobwhite exposed to 2,4-D in the diet was therefore 1000 ppm, the highest concentration tested.

Neurotoxicity

Test Substance:	**2,4-D**
Report:	Chittibabu, 2002d
Guidelines:	Gaitonde Committee Guideline G.3.0.Ci
GLP:	No (Quality Assurance Statement provided)

Test System

A non-standard test was conducted to determine the neurotoxicity of 2,4-D Acid technical in egg laying chickens (*Gallus domesticus*) as an experimental model, when administered as multiple doses for 21 days. Four groups were used for the test, each consisting of 26 birds. Birds were acclimated to laboratory conditions for 7 days, and were 1.4-1.6 kg of weight at the start of the experiment. They were housed individually in layer cages with wire meshed floors and provided with feed and water troughs and dropping trays. A 12 hour light:dark photoperiod was maintained. Both water and feed were given *ad libitum*.

Based on the results of a pilot study (see Chittibabu, 2002a above), three doses of 25, 50 and 100 mg/kg bw were selected as test dose levels ($1/40^{th}$, $1/20^{th}$ and $1/10^{th}$ of the LD50 respectively). All birds were starved overnight prior to initial dosing. The test substance was delivered in vegetable oil and a control group was maintained. Doses were administered by intubation. Dosing was continued daily for 21 days and on day 22, half the birds from each group were sacrificed for gross pathological evaluation and the remaining birds observed for a further 21 days during which no further doses were administered. At the end of this period all birds were sacrificed for gross pathological evaluation.

Mortality and general health was observed daily. To detect ataxia, every day the birds were removed from the cages and dropped from a height of around 4 ft and allowed to move a few yards on the ground for detection of locomotor ataxia and paralysis. Haematology was assessed through drawing blood samples on days 0, 22 and 43. Additionally, effects on plasma were investigated. Body weights of individual birds were recorded prior to test initiation and daily during the entire observation period. Egg weight and egg yield of each bird along with feed consumption were recorded daily during the entire observation period.

Changes in body weight, feed consumption, haematology and biochemistry of birds were compared to the control group using Student's t-test.

Findings:

There were no significant changes in body weight, feed consumption, egg yield or egg weight in treated groups of birds when compared with that of control birds over the observation period.

There were no remarkable changes in haematological and biochemical parameters in treated groups of birds when compared with that of control birds.

No ataxia was seen in treated groups of birds throughout the observation period with the exception of two birds in the 100 mg/kg bw group groups, which exhibited lethargy and incoordination. These symptoms were first noticed in day 8. One of the birds was in the group sacrificed at day 22. The other bird continued to show these signs for five days into the post-exposure observation period. After day 26, this bird had returned to normal.

No other overt signs of toxicity were recorded. No mortality was observed in the test substance treated groups and control groups of birds throughout the observation period.

Conclusion:

Although haematological, biochemical and histopathological evaluations did not reveal any abnormality, based on observations of overt signs of toxicity in the highest treatment level, the NOEL of 2,4-D Acid to chickens under the conditions of this study can be considered to be 50 mg/kg bw.

Conclusions for Avian Toxicity

When tested in its acid form, 2,4-D was not toxic to birds based on acute oral exposure from one standard test (not reviewed) and two non-standard tests. Short term toxicity was not evident with three tests all resulting in an undefined LD50 due to a lack of mortality at up to 5620 ppm (although 40% mortality at this level was found for the bobwhite quail). A single reproduction test showed no effects on reproductive parameters for the bobwhite quail at 1000 ppm. There was no corresponding reproduction study for the mallard duck. However, given the lack of effects through dietary exposure for both species, and the lack of effects on bobwhite quail through the reproduction study, this test is not considered necessary.

Aquatic Toxicity

Fish – Acute

The APVMA received 7 acute fish toxicity tests with the following results:

Table A1.27. Summary of Acute Fish Toxicity Results for 2,4-D Acid

Test species	System	LC50 (mg/L)	Reference
Rainbow trout (*S. gairdneri*)	96 h flow-through	320	Alexander *et al*, 1983a
Bluegill sunfish (*L. macrochirus*)	96 h flow-through	263	Alexander *et al*, 1983a
Fathead minnow (*P. promelas*)	96 h flow-through	358	Alexander *et al*, 1983a
Tidewater silverside (*M beryllina*)	96 h flow-through	175 (m)	Vaishnav *et al*, 1990a
Guppy (*P. reticulata*)	96 h static	>105	Chittibabu, 2002k
Zebra fish (*B. rerio*)	96 h static	>105	Chittibabu, 2002k
Mozambique tilapia (*T mossambica*)[1]	96 h static	>100[1]	Chittibabu, 2002l

1) non-standard test; m = measured concentration

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

Report: Alexander *et al*, 1983a

Guidelines: US EPA Guideline 72-1

GLP: no

Test system:

The acute toxicity of 2,4-D 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 10 litres of dilution water to which an appropriate amount of weighed test material was added. Vessels were heated and stirred to help dissolve the 2,4-D. Vessels were aerated prior to the addition of 10 fish, which initiated the test. Fish were not fed during the test.

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 7.8-7.9, hardness of 102-108 mg/L as $CaCO_3$ and alkalinity 79-83 mg/L as $CaCO_3$. Nominal test concentrations for all species were 0 (control), 204, 256, 320, 400 and 500 mg/L.

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 and these are highlighted below.

Findings:

No mortality was recorded in any of the controls used for any of the different species. In the fathead minnow test, dissolved oxygen ranged from 7.6-10.0 mg/L while temperature was around 17^{o}C. The bluegill sunfish test had a dissolved oxygen range from 7.0-10.2 mg/L while temperature was around 17^{o}C. The rainbow trout test had a dissolved oxygen range from 7.0-10.2 mg/L while temperature was around 12^{o}C

2,4-D affected the pH of the test solutions. For all three species, the pH in the 400 and 500 mg/L groups at the start of the test was around 3.8 and 3.3 respectively. All fish in these concentrations were dead after 24 hours in the fathead minnow and bluegill sunfish study. Only 96 h observations are available for rainbow trout, but 100% mortality at both these concentrations is recorded after 96 h with no mortality at lower concentrations. No pH data are recorded in the test report for other concentrations except that in the fathead minnow study, the 320 mg/L group had an initial pH of 5.9 and this had increased to 7.0 after 24 hours.

Mortality figures for the three species after 96 hours were reported as follows:

Table A1.28: Mortality data for Fathead Minnow, Bluegill Sunfish and Rainbow Trout

mg/L	Fathead minnow %mortality	Bluegill sunfish %mortality	Rainbow trout %mortality
0	0	0	0
204	0	30	0
256	0	30	0
320	50	70	0
400	100	100	100
500	100	100	100

Conclusion:

The LC50s with corresponding 95% confidence intervals are shown below.

	LC50 mg/L	95% CI (mg/L)	Statistical method
Fathead Minnow	320	299-343	Moving average
Bluegill	263	220-302	Probit
Rainbow Trout	358	320-400	Binomial

Report: Vaishnav *et al*, 1990a

Guidelines: US EPA FIFRA Guideline 72-3

GLP: yes

Test system:

The acute toxicity of 2,4-D was tested on tidewater silverside (*Menidia beryllina*) under flow-through conditions for 96 hours. Concentrations tested were control, 104, 173, 288, 480 and 800 mg/L. 20 fish per concentration (2 replicates of 10) were used in the experiment. Dilution water consisted of filtered seawater diluted to a salinity of 20 ppt. Flow-through conditions was such that there were 4.6 daily volume additions to each exposure chamber.

The test was conducted using 30 L glass aquaria with 18 L dilution water. They were kept covered during the test. Test conditions included water temperature of around 22°C; 16:8 hours light: dark; pH range 8.1-8.3 (control water) and dissolved oxygen of 8.0-8.9 mg/L (control water). Water samples were taken from the solvent control and each test concentration (duplicates pooled) at 0, 24 and 96 hours for analysis.

All aquaria were examined daily for mortality and behavioural changes. Dead organisms were removed. Water quality parameters were measured daily in each exposure vessel until test termination or 100% mortality. At test initiation, water samples from all exposure chambers and the stock solution were analysed for 2,4-D. Additional water samples were analysed at 48 and 96 h, or when 100% mortality had occurred in any of the exposure chambers.

The 96 h LC50 was calculated statistically using the moving average method.

Findings:

Mean measured concentrations ranged from 111 ppm in the lowest treatment concentration to 743 ppm in the highest. Water quality measurements were all within acceptable limits with the exception of pH in the highest exposure group. Average pH in all other groups including the control ranged from 8.0-8.2, however, in the highest

group, the median pH was measured at 5.5 indicating higher 2,4-D acid concentrations impacted pH.

The following mortality was observed (measured concentrations reported):

Table A1.29: Cumulative mortality of Tidewater Silverside

Measured concentration (mg/L)	Cumulative mortality (%)			
	24 h	48 h	72 h	96 h
0	0	0	0	0
111	0	5	30	40
167	0	15	40	50
285	0	15	45	55
461	35	45	100	100
743	100	100	100	100

Conclusions:

The 96 h LC50 for 2,4-D using tidewater silverside was determined to be 175 mg/L (measured) with 95% confidence limits of 128-217 ppm. A NOEC could not be determined due to significant mortality at the lowest concentration tested.

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

Report: Chittibabu, 2002k

Guidelines: OECD Test Guideline 203

GLP: Yes

Test System

Acute toxicity of 2,4-D Acid Technical was tested on two freshwater fish, the Guppy (*Poecilia reticulata*) and Zebra fish (*Brachydanio rerio*) in a static test. The test was performed over 96 hours under a 12 h light:dark photoperiod. Fish were acclimated to laboratory conditions for 7 days with feeding stopped 24 h prior to test initiation.

Water was analysed for pH, temperature, dissolved oxygen and total hardness at the end of every 24 hours during the exposure period. The test substance was dissolved in methanol to prepare stock solutions. Samples from the exposure media were checked for stability and homogeneity and found to be within permissible limits (although these results were not reported).

Based on the results of a range finding test, groups of fish (10 per group, 1 replicate per treatment) were exposed to 10, 18, 32.4, 58.3 and 105 mg/L (nominal) along with a solvent control group. It appears no standard dilution water control group was maintained.

Observations for mortality and abnormal behaviour was made at 3 and 6 h, and thereafter, every 24 h until 96 h.

Findings:

Throughout the test, pH ranged from 7.1-7.3, temperature from 20-21^{o}C, dissolved oxygen from 7.7-7.9 mg/L and total hardness as $CaCO_3$ from 265-282 mg/L.

No mortality or behavioural abnormalities were observed in either test species throughout the test.

Conclusion:

The 96 h LC50 for both the guppy and zebra fish is >105 mg/L. The NOEC was the highest level tested of 105 mg/L.

Test Substance:	**2,4-D**
Report:	Chittibabu, 2002l
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 Acid Technical 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 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 dissolved in methanol 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/L (nominal) along with a solvent control group. It appears no standard dilution water control group was maintained. 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 parameters, pH was 7.1, temperature was 22^{o}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/L. The NOEC was the highest level tested of 100 mg/L.

Fish - Chronic/Sub-Chronic

One study was provided to the APVMA for review.

Report:	Mayes *et al*, 1990c
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 32 days. Embryos less than 24 h old were used for the test. The test was started by impartially distributing 20 embryos to each

embryo cup (80 per treatment). Measured exposure concentrations were 0 (control), 12.6, 22.2, 37.4, 63.4 and 102 mg/L.

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 29 days post day-to-mean hatch of the controls (32 days total). At test termination, all surviving fish were measured for weight and length.

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

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

Findings:

Temperature in all test vessels ranged from 25.0-25.6^{o}C throughout the study. Dissolved oxygen concentrations ranged from 7.9-8.6 mg/L in all vessels throughout the study. There was a decrease in pH of dilution water as exposure concentration increased. In the control, the pH ranged from 7.5-7.7 compared to a range of 6.5-6.9 in the 102 mg/L exposure group.

Biological results are summarised in Table 22 below.

Table A1.30: ELS toxicity (32 d) of 2,4-D on Fathead Minnow

Concentration [µg ai/L]	Control	12.6	22.2	37.4	63.4	102
Embryos hatched (%)	100	100	100	100	98.75	98.75
Normal Larvae at Hatch (%)	100	100	100	98.75	96.25	97.50
28 day Larval Survival (%)	93.75	96.25	93.75	96.25	90.00	82.50*
Mean Wet Weight (mg)	47.05	53.43	62.49	65.80	63.71	48.99
Mean Length (mm)	14.82	15.62	16.23	16.55	16.34	14.78

* - Statistically different to the control.

There was a statistically significant reduction in survival when compared to the controls at 102 mg/L treatment level on days 8 through 32. Survival was also statistically reduced on days 5 and 14 in the 63.4 mg/L treatment level; these data are inconsistent with other effect observations and are judged to have no toxicological significance.

Conclusion

The NOEL was 63.4 mg/L. The MATC was calculated to be 80.4 mg/L based on the LOEC of 102 mg/L.

In their 1989 report, WHO (1989) makes the following appraisal with respect to toxicity to fish:

> At recommended application rates, the concentration of 2,4-D in water has been estimated to be a maximum of 50 mg/litre. Most applications would lead to water concentrations much lower than this (between 0.1 and 1.0 mg/litre). LC50 values for fish vary considerably. This variation is due to differences in species sensitivity, chemical structure (esters, salts, or free acid), and formulation of the herbicide. Although the free acid is the physiologically toxic entity, the ester formulations represent a major hazard to fish when used directly as aquatic herbicides (because they are more readily taken up by fish). Amine salt formulations used to control aquatic weeds do not affect adult fish.
>
> The NOEL varies with the species and the formulation: <1 mg/litre (coho salmon) to 50 mg/litre (rainbow trout).
>
> Fish larvae are the most sensitive life stage but are unlikely to be affected under normal usage of the herbicide.
>
> Long-term adverse effects on fish are observed only at concentrations higher than those produced after 2,4-D has been applied at recommended rates.
>
> Few studies are related to the effects of environmental variables, such as temperature and water hardness, on 2,4-D toxicity to fish. Higher temperature possibly increases the toxicity. This might be considered when assessing the safety of 2,4-D to fish during control of aquatic weeds.
>
> Fish detect and avoid 2,4-D only at higher concentrations than those obtained under normal conditions of use.

The current review of 2,4-D aquatic toxicity is consistent with these statements. The conclusions from this review are summarised below (end of aquatic toxicity section).

Amphibians

One amphibian toxicity test was provided to the APVMA for review.

Test Material:	**2,4-D**
Report:	Palmer and Krueger, 1997a
Guidelines:	Full protocol provided
GLP:	Yes

Test system:

Acute toxicity of 2,4-D 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), 65, 108, 180, 300 and 500 mg/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 53 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 30 mg. Loading was calculated to be 0.18 g tadpole/L.

Test chambers were 2 L glass beakers containing around 1.8 L test solution with a water depth around 13.5 cm. Dilution water consisted of well water passed through a sand filter with further filtering to remove micro-organisms and fine particles. The test was performed with a 16 h light per day photoperiod and a 30 minute dawn/dusk transition period. Temperature was measured continuously in one negative replicate. Dissolved oxygen and pH measurements were made in water sampled in all test chambers daily. Hardness and alkalinity were measured in the highest exposure group and control group at test initiation.

Organisms were observed for mortality and behaviour at 3.5, 24, 48, 72 and 96 hours. The 96 h LC50 was calculated by probit analysis.

Findings:

Mean measured concentrations ranged from 100-105% nominal, and the measured exposure concentrations were 65, 113, 186, 313 and 510 mg/L. Temperature ranged from 21.5-22.5^{o}C and dissolved oxygen from 7.6-8.6 mg/L at the start of the study to 5.9-6.5 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 128 and 178 mg/L as $CaCO_3$ respectively.

Mortality data were reported as follows:

Table A1.31: Cumulative Mortality Data for Tadpoles

Exposure (mg/L)	Cumulative mortality (%)			
	24 h	48 h	72 h	96 h
0	0	0	0	0
65	0	0	0	0
113	0	0	0	0
186	0	0	0	0
313	0	15	25	50
510	35	60	60	75

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 was 359 mg/L (95% CI 308-428 mg/L). Based on the mortality and observation data, the 96 h NOEC was 186 mg/L.

In addition, WHO (1989) reports that tadpoles of the Indian toad are particularly susceptible to 2,4-D. The result reported for the free acid was a 96 h LC50 of 8.3 mg/L. It is also reported that 2,4-D, at 5 g/litre, prevented development of the eggs of the common frog (Rana temporaria). At doses between 500 mg/litre and 4 g/litre, there was some development that decreased with increasing dose.

Invertebrates - Acute

Table A1.32. Summary of Acute Aquatic Invertebrate Toxicity Results for 2,4-D Acid

Test species	System	LC50 (mg/L)	Reference
Daphnia magna	48 h static	247.2	McCarty and Batchelder, 1977
Daphnia magna	48 h static	74.4	Chittibabu, 2002p
Daphnia magna	48 h static	262	McCarty, 1979
Daphnia magna	48 h static	25.0	Alexander *et al*, 1983c
Daphnia magna	48 h static	36.4	Alexander *et al*, 1983c
Eastern oyster (*C. virginica*)	96 h flow-through	EC50 = 57	Ward e*t al*, 1979
Eastern oyster (*C. virginica*)	96 h flow-through	EC50 = 146	Wade and Overman, 1990
Pink shrimp (*P. duorarum*)	96 h flow-through	554	Vaishnav *et al*, 1990

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

Report: McCarty and Batchelder, 1977

Guidelines: US EPA Guideline 72-2

GLP: no

Test system:

Acute toxicity of 2,4-D to *Daphnia magna* Straus, was evaluated under static conditions. The water used for the study was dechlorinated Lake Huron water with dissolved oxygen of 7.7 mg/L, pH of 7.6, total alkalinity of 85 mg/L as $CaCO_3$ and total hardness of 100 mg/L as $CaCO_3$.

First instar daphnids were exposed to nominal concentrations of 155, 180, 210, 240, 280 and 320 mg/L for a period of 48 hours at 25^{o}C water temperature. It does not appear that analysis was undertaken of the test water through the course of the study to verify dosing rates. 10 daphnids were added to each test beaker. Each concentration had three replicates (30 daphnids per concentration), as did the control group. Mortality data were recorded at 24 and 48 hours.

The LC50 was determined using probit analysis.

Findings:

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

Table A1.33: Mortality findings for *Daphnia Magna*

Concentration (mg/L)	% Mortality	
	24 hours	48 hours
155	0	0
180	0	7
210	3	13
240	30	43
280	67	77
320	93	93

No further observations are reported in the test report.

Conclusions:

The 48 h LC50 was calculated using probit analysis to be 247.2 mg/L (95% CI = 236.6-258.8 mg/L). Based on the above table, the 48 h NOEC can be defined as 155 mg/L.

Test Substance:	**2,4-D**
Report:	Chittibabu, 2002p
Guidelines:	OECD Test Guideline 202
GLP:	Yes

Test System

Acute toxicity of 2,4-D Acid Technical was tested on *Daphnia magna* over 48 h in a static test. Daphnids 24 h old or less were used for the experiment and not fed throughout the test. The test was performed under controlled temperature and humidity (values not reported) using a 12 h light:dark photoperiod. Test substance was dissolved in methanol for making stock solutions.

Daphnids were exposed in 4 batches, each with 5 animals, to each test concentration and a solvent control. The test concentrations were 50, 60, 72, 86.4 and 103.7 mg/L (nominal) and were chosen based on a range finding study. It does not appear that any analysis was performed to verify test concentrations.

At the end of 48 h, the number of daphnids immobilised in test concentrations and the control were counted. Water quality parameters were measured at the start and end of the test. The EC50 was calculated using probit analysis.

Findings:

Throughout the test (as measured at the beginning and end), pH ranged from 7.2-7.4, temperature from 20-21^{o}C, dissolved oxygen from 7.8-7.9 mg/L and total hardness as $CaCO_3$ from 265-271 mg/L.

Based on a total number of 20 daphnids exposed to each concentration, mean immobilisation in the control, 50, 60, 72, 86.4 and 103.7 mg/L groups was 0, 0, 20, 40, 70 and 100% respectively after 48 hours.

Conclusion:

The 48 h EC 50 of 2,4-D acid technical for *Daphnia magna* was calculated as 74.4 mg/L with confidence limits (assumed to be 95%) of 71.8-77.1 mg/L. The NOEC was not stated by the study authors, but based on immobilisation, can be considered as 50 mg/L.

Test Material:	**2,4-D**
Report:	McCarty, 1979
Guidelines:	US EPA Guideline 72-2
GLP:	no

Test system:

2,4-D was among 11 herbicides tested for acute toxicity to *Daphnia magna* Straus, under static conditions. The water used for the study was dechlorinated Lake Huron

water with dissolved oxygen of 7.7 mg/L and total hardness of 100 mg/L as $CaCO_3$. Total alkalinity and pH are not reported.

First instar daphnids were exposed to nominal concentrations of 155, 180, 210, 240, 280 and 320 mg/L for a period of 48 hours at 20°C water temperature. It does not appear that analysis was undertaken of the test water through the course of the study to verify dosing rates. 10 daphnids were added to each test beaker. Each concentration had three replicates (30 daphnids per concentration), as did the control group. Mortality data were recorded at 24 and 48 hours.

The LC50 was determined using the moving average method.

Findings:

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

Table A1.34: Mortality findings for *Daphnia Magna*

Concentration (mg/L)	% Mortality	
	24 hours	48 hours
155	7	10
180	7	13
210	10	27
240	7	30
280	37	50
320	100	100

No further observations are reported in the test report.

Conclusions:

The 48 h LC50 was calculated using to be 262 mg/L (95% CI = 248-276 mg/L).

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

Report: Alexander *et al*, 1983c

Guidelines: US EPA Guideline 72-2

GLP: no

Test system:

The acute toxicity of 2,4-D was tested on *Daphnia magna* over 48 hours using a static test system. Two tests were run, one with Lake Huron water, and the other with dilution water. 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 18 psi for 35 minutes. Water quality parameters for the daphnid dilution water during the course of the study were pH of 7.9-8.2, hardness of 143-157 mg/L as $CaCO_3$ and alkalinity 53-62 mg/L as $CaCO_3$. This compared with the Lake Huron water with pH of 7.8, hardness of 102-108 mg/L as $CaCO_3$ and alkalinity 79-83 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 to dilution water were control, 12, 18, 28, 42, 65 and 100 mg/L, with 10 daphnids exposed to each group in triplicate (30 animals total per group). Exposure concentrations to Lake Huron water were control, 10, 20, 42, 65 and 100 mg/L.

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 groups throughout the study. In the dilution water study, dissolved oxygen concentration ranged from 8.8-9.4 mg/L and the pH remained between 7.0-8.1. This compared to dissolved oxygen range of 8.5-9.3 mg/L and pH of 7.0-8.2 in the Lake Huron water. Mortality findings for the controls and test concentrations are as follows:

Table A1.35: Mortality Findings for *Daphnia magna*.

Concentration (mg/L)	% Mortality (Dilution Water)		Concentration (mg/L)	% Mortality (Lake Huron Water)	
	24 hours	48 hours		24 hours	48 hours
Control	0	0	0	0	0
12	0	20	10	0	17
18	0	37	20	7	47
28	0	70	42	13	53
42	0	77	65	27	87
65	3	67	100	7	73
100	10	80			

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

Conclusion:

The 48 h LC50s were calculated by probit analysis. The 48 h LC50 in the dilution water was calculated to be 25.0 (95% CI 17.6-32.6) mg/L. In the more buffered Lake Huron water, the 48 h LC50 was 36.4 (95% CI 28.2-45.4) mg/L. These results are not considered statistically different.

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

Report: Ward *et al*, 1993.

Guidelines: US EPA Guideline 72-3(c)

GLP: Yes

Test system:

Acute toxicity of 2,4-D was tested on the Eastern oyster (*Crassostrea virginica*) in a 96 h flow-through test, judged by the deposition of new shell. Oysters were around 12 months old at the test initiation. They were acclimatised for 16 days during which time the temperature ranged from 22 to 23.8^{o}C, salinity ranged from 33-34 ppt, and dissolved oxygen was at least 6.3 mg/L. Oysters were 25-45 mm in height (long axis) at test intiation.

Dilution water consisted of natural unfiltered seawater where it was recirculated in the laboratory prior to use. The water had a pH of 7.8 and salinity of 32 ppt. Test aquaria maintained a test solution volume of around 15 L and were equipped with an intermittent flow proportional diluter set to deliver approximately 8.1 volume replacements every 24 hours and 0.51 L per oyster per hour. At initiation, each oyster was ground to remove around 3-5 mm of shell and form a smooth edge. Exposure was initiated by impartially selecting and placing 20 oysters in each test aquarium (40

per treatment level and the controls). Test conditions included a temperature of around 21-22°C and a 16:8 hour light:dark photoperiod.

Concentrations tested included a dilution water control, 29, 48, 76, 110 and 190 mg/L. The number of surviving organisms and the occurrence of sub-lethal effects were determined visually and recorded after 0, 24, 48, 72 and 96 hours. At the end of the study oysters were removed from test vessels and the longest finger of new growth was measured to the nearest 0.1 mm.

Analytical determination of 2,4-D concentration was performed on pooled samples from the 2 replicates of each concentration at 0, 48 and 96 h. The EC50 calculations were performed with mean measured concentrations using the moving average method. The NOEC was calculated using Dunnett's test.

Findings:

Insoluble material was not noted in the test vessels during the test. The mean measured concentrations of 2,4-D were 0, 30, 50, 78, 120 and 200 mg/L in the treatment groups. These ranged from 103-109% of nominal concentrations and were stable throughout the test. The pH was affected by the test substance resulting in slightly lower pH values at higher concentrations. Nonetheless, pH values for all concentrations and the control group ranged from 7.0-8.0.

In the control group, survival was 100% and no sub-lethal effects were observed throughout the study. Control oysters deposited an average of 2.7 mm of new shell during the 96 h exposure period.

In the treatment groups, mortality was 0% in the 30, 50 and 78 ppm groups and increased to 35% and 85% in the 120 and 200 ppm groups respectively. Faeces production was visibly reduced in the two higher concentration test vessels. No other sub-lethal effects were observed. Shell growth by oysters averaged 2.5, 1.6, 0.8, 0 and 0 mm in the 30, 50, 78, 120 and 250 mg/L groups respectively compared to the average 2.7 mm in the control group. Only growth in the lowest test group is not considered to be statistically significantly different from the control.

Conclusions:

The 96 h EC50 was calculated to be 57 mg/L with a 95% CI of 54-60 mg/L. The 96 h NOEC was 30 mg/L 2,4-D. The report does not calculate an LC50. Considering the decline in survival from 78-200 ppm, an LC50 of around 127 ppm is estimated using LN(% survival) and plotting against concentrations in Excel.

Test Material:	**2,4-D**
Report:	Wade and Overman, 1990
Guidelines:	US EPA Guideline 72-3
GLP:	Yes

Test system:

Acute toxicity of 2,4-D was tested on the Eastern oyster (*Crassostrea virginica*) in a 96 h flow-through test, judged by the deposition of new shell. Oysters between 28.5-39.8 m in length were maintained for 55 days prior to test initiation in full strength, unfiltered seawater and were not given additional food during this time.

Dilution water consisted of unfiltered seawater. The test was conducted in glass exposure chambers filled with 13.4 L dilution water using a continuous flow diluter system. The flow rate was set at 1.05 L/oyster/hour. Nominal concentrations tested were 0 (control), 104, 173, 288, 480 and 800 mg/L. 20 oysters were distributed to each of the control and treatment chambers. It is assumed their shells were ground to create a smooth edge prior to test initiation in accordance with the guideline. A 16 h light per day photoperiod was maintained with a 30 minute dawn/dusk transition period, and a water bath set to regulate water temperature of around 20°C.

Test organisms were observed daily for any mortality and behavioural changes, and dead organisms removed. Water quality parameters were measured daily in each exposure chamber or until 100% mortality occurred. Salinity was measured daily in control chambers and test temperature was recorded hourly in a control chamber. Water samples were analysed for actual exposure concentration at the start of the test, and again at 48 and 96 hours or when 100% mortality occurred.

The EC50 and 95% confidence limits were determined by non-linear regression.

Findings:

Based on exposure water analysis, mean measured exposure concentrations were 0, 135, 183, 294, 449 and 672 mg/L. Temperature was a steady 21°C from 24 hours onwards (20°C at the 0 h measurement), dissolved oxygen ranged from 6.5-7.2 in all vessels over the course of the study, and salinity ranged from 36-37 ppt. Exposure levels had an impact on pH. The pH range in control chambers was 8.0-8.1 over the study. This compared to 7.9-8.1; 7.6-7.7; 7.2-7.7; 6.7-7.5; and 3.7-3.9 in the 135, 183, 294, 449 and 672 mg/L exposure groups respectively.

The three highest treatment levels resulted in complete mortality of oysters. The full mortality and reduction in shell growth data were reported as follows:

Table A1.36. Cumulative Mortality and Effects on Shell Growth

Exposure (mg/L)	Cumulative mortality (%)				% reduction in shell growth
	24 h	48 h	72 h	96 h	
0	0	0	0	0	0
135	0	0	0	5	32
183	0	0	5	15	90
294	0	0	90	100	100
449	0	85	100	100	100
672	20	85	100	100	100

Conclusions:

The 96 h EC50 was calculated to be 146 mg/L with a 95% CI of 136-157 mg/L. A NOEC could not be estimated due to significant reduction in shell growth at the lowest concentration tested.

The test authors did not calculate an LC50. However, based on the above data, it would appear to be between 183 and 294 mg/L, and probably due to increased acidity of the test medium at the higher concentrations.

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

Report: Vaishnav *et al*, 1990

Guidelines: US EPA Guideline 72-2

GLP: Yes

Test system:

The acute toxicity of 2,4-D was tested on the Pink shrimp (*Penaeus duorarum*) over 96 hours under flow-through conditions. Shrimp were between 79 and 98 mm in length. Dilution water was filtered seawater adjusted to around 20 ppt and a 16 h light photoperiod with 30 minute dawn/dusk transition was maintained. Temperature was maintained between 21 and 23^{o}C.

The test was performed in 30 L aquaria, filled to 18 L with dilution water. They were kept covered during the test. It is unclear how many volume replacements occurred daily. Nominal concentrations were 0 (control), 104, 173, 288, 480 and 800 mg/L. One day prior to test initiation, samples of test substance stock solution and exposure water from all chambers were analysed for 2,4-D. Additional water samples were analysed at 48 and 96 h or when 100% mortality occurred.

10 shrimp were assigned to duplicate vessels (20 per treatment) for all exposure levels and the control. They were observed daily for mortality and behavioural changes and dead animals were removed. Water quality parameters were measured daily and test temperature was measured hourly.

The 96 h LC50 was calculated using the binomial method.

Findings:

Mean measured concentrations ranged from 102-108% of nominal for all concentrations tested. Mortality data below are provided in terms of measured concentrations. Temperature measurements ranged from 21.1-22.6^{o}C throughout the study. Dissolved oxygen ranged from 5.3-8.8 mg/L in all test vessels throughout the study. Addition of 2,4-D had an impact on pH. In the control and 104 and 173 mg/L concentration groups, pH ranged from 7.7-8.1 over the study. However, the ranges in the 288, 480 and 800 mg/L groups decreased with increasing concentration and were 7.3-8.1, 6.9-7.8 and 3.6-4.5 respectively over the course of the study (only determined up to 24 h in the highest exposure group due to full mortality).

Mortality data are shown as follows:

Table A1.37: Cumulative Mortality Results for Pink Shrimp

Measured concentration (mg/L)	Cumulative percent (%) mortality.			
	24 h	48 h	72 h	96 h
0	0	0	0	5
107	0	0	0	5
187	5	5	5	5
296	25	25	25	25
495	20	25	25	30
815	100	100	100	100

No abnormal behavioural observations were recorded in the test report.

Conclusion:

The 96 h LC50 for pink shrimp was determined to be 554 mg/L 2,4-D with 95% confidence limits of 296 and 815 mg/L 2,4-D. A NOEC was estimated to be 187 mg/L.

Invertebrates – Chronic

One chronic aquatic invertebrate study was provided to the APMVA as follows:

Report: Ward and Boeri, 1991g

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 using a flow-through system. Animals were 24 h old or less at the commencement of the study. They were acclimated in well water with a hardness of 160-180 mg/L as $CaCO_3$. Based on the results of preliminary testing, definitive test exposure concentrations were 0, 112, 192, 320, 472 and 800 mg/L.

A photoperiod of 16:8 h light:dark was maintained. The system was fitted with an intermittent flow proportional diluter designed to deliver around 13.3 volume replacements every 24 h. Each vessel contained 10 daphnids with four replicates (40 animals) per treatment level and control.

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

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

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

Results were interpreted by standard statistical techniques. All calculations were performed using mean measured concentrations. The 21 Day EC50 was calculated by non-linear interpolation.

Findings:

Water quality parameters were within acceptable levels throughout the test. Measured concentrations of dissolved oxygen were always above 60% saturation (5.6 mg/L); the temperature ranged from 19.0-21.0^{o}C and pH ranged from 4.0-8.5 (values below 7.7 occurred only at the highest tested concentration).

Measured concentrations ranged from 70-86% of nominal values, and actual exposure concentrations were 0, 79, 151, 258, 388 and 689 mg/L.

Only the 21 day survival of first generation adult daphnids was statistically analysed. The number of young produced, the average number of young per female and the weight of surviving daphnids were greater than the control at the concentration of test substance that was shown not to differ statistically from the control in 21 day survival. Sub-lethal effects were never observed in test vessels containing the concentration of 2,4-D that did not differ from the control in 21 day survival, and the time to first brood was identical to the control in this treatment.

Table 24 below summarises the biological results from the definitive experiment.

Table A1.38: Effects of 2,4-D on *Daphnia magna* in a Chronic Reproduction Study at 21 Days

Measured concentration [mg/L]	Control	79	151	258	388	689
Parent immobilisation (%)	92.5	90.0	82.5	42.5	0	0
Total mean offspring	934	951	7	-	-	-
Mean young/surviving female	101	106	1	-	-	-
Mean body weight (mg)	0.62	0.65	0.19	-	-	-

The percentage of surviving adults was significantly reduced in comparison to the control at all concentrations above 151 mg/L and the average dry weight of surviving adult daphnids was significantly less than the control at concentrations above 79 mg/L. The total number of young produced and the mean number of young per surviving female was greater than the control at 79 mg/L and no sub-lethal effects were noted during the test in the control or at the 79 mg/L treatment level.

Conclusions:

Exposure of daphnids to 2,4-D for 21 days resulted in a MATC of 109 mg/L, calculated as the geometric mean of the NOEC (79 mg/L) and the LOEC (151 mg/L). The 21 day median EC50 was 235 mg/L (95% CI of 151-383 mg/L).

Algae and aquatic plants

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

Table A1.39. Summary of Algae/Aquatic Plant Toxicity Results for 2,4-D Acid

Test species	System	EC50 (mg/L)	Reference
Green alga *(S. capricornutum)*	120 h	EC25 = 29.0 EC50 = 33.2	Hughes, 1990a
Green alga (*C. vulgaris*)	72 h	101.5	Chandrasehar, 2001

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

Report: Hughes, 1990a

Guidelines: US EPA Guideline 123-2

GLP: yes

Test system:

Growth inhibition resulting from exposure to 2,4-D by the freshwater green algae, *Selenastrum capricornutum* was investigated over 120 hours. Nominal concentrations tested were 6.19, 12.4, 24.8, 49.5 and 99.1 mg/L. Additionally, a solvent, N,N-dimethylformamide (DMF – used in making the stock solution) at 0.5 mL/L and a blank control were tested.

An algal inoculum was prepared from a 7-day old stock culture. A 0.158 mL volume of this sample was added to 50 mL of medium in each of three replicate flasks per test treatment, yielding nominal initial concentrations of 3000 cells/mL. Flasks were incubated at a temperature of around 24^{o}C with continual shaking at 100 oscillations per minute. Continuous illumination was provided.

Cell counts were made on test days 3, 4 and 5. Three counts per replicate were made. Samples were analysed for actual concentration on 2,4-D on days 0 and 5. At test termination, the contents of the replicate flasks were combined, the pH recorded and the contents filtered. The filtrates were analysed using HPLC.

The NOEC was determined from an analysis of variance and Tukey's test. The level of significance was 0.05.

Findings:

Recoveries of measured concentrations were 70-133% of nominal values. Actual exposure concentrations based on mean measured day 0 and 5 values were 4.76, 11.7, 26.4, 59.8 and 112.5 mg/L. The pH was affected by the addition of 2,4-D. At the end of the study, the pH in the control, solvent control, 4.76, 11.7, 26.4, 59.8 and 112.5 mg/L exposure groups was 7.45, 7.55, 7.80, 7.80, 8.10, 4.80 and 4.10 respectively.

Inhibition of growth as compared to the solvent control is shown as follows:

Exposure (mg/L):	Control	4.76	11.7	26.4	59.8	112.5
% inhibition:	20	29.0	13.7	24.4	100	100

While similar effects on growth were seen in the 4.76, 11.7 and 26.4 mg/L groups, the two highest concentrations proved toxic to the algae with complete inhibition. On day 5 at these two highest concentrations, around 1,333-1,667 cells/mL were obtained compared to around 3000 cells/mL on day 0. This compared to some 4.4 million cells/mL found in the solvent control at day 5.

Conclusion:

As determined by weighted least squares non-linear regression, the 5 day EC25 is 29.0 mg/L (95% CI 0.01-81,283 mg/L) and the 5 day EC50 is 33.2 mg/L (95% CI 0.02-52,119 mg/L). It is reported that confidence limits are so wide because there were no data points in the mid-range of the dose-response.

It is likely that at the two higher concentrations, effects are acid induced rather than toxicity of 2,4-D itself.

Test Material:	**2,4-D**
Report:	Chandrasehar, 2001
Guidelines:	OECD Test Guideline 201
GLP:	Yes

Test system:

Growth inhibition resulting from exposure to 2,4-D acid technical by the freshwater green algae, *Chlorella vulgaris* was tested under *in vitro* conditions. The test was performed in a growth cabinet over three days (72 hours) with continuous illumination and a temperature range of 19.5-22.5^{o}C.

The alga was cultured in a nutrient medium recommended by the test guideline. The medium was sterilised and uniformly distributed into 250 mL conical flasks, each containing around 150 mL. The stock solution was prepared by dissolving the test substance in acetone. Treatments for the study were control, 1.0, 5.0, 15.0, 25.0, 50.0, 75.0, 100.0 and 150.0 mg/L (nominal) and were based on the results of a range finding test. Three replicates per treatment were performed.

The growth was assessed by counting cells at 24, 48 and 72 h after inoculation, using improved Neubauer haemocytometer under binocular photomicroscope.

Findings:

Increasing 2,4-D concentration had an affect on the pH of the test medium. At the start of the test, pH in all media was reported as 8.0. After 72 hours, the mean pH of the three replicates for the various treatments was between 10.56-10.95 in the control and up to 50 mg/L. At the next three treatment levels of 75, 100 and 150 mg/L, pH declined to 9.71, 8.53 and 6.44 respectively.

Growth was impaired in a definite dose response fashion. At the end of 72 hours, when compared with the control growth, inhibition in the 1.0, 5.0, 15.0, 25.0, 50.0, 75.0, 100.0 and 150.0 mg/L treatment levels was 2.22, 7.27, 15.28, 21.57, 27.58, 35.71, 44.87 and 78.52% respectively.

Conclusion:

The EC50 was calculated using regression analysis (log concentration vs % growth inhibition). The 72 h EC50 was calculated to be 101.5 mg/L (nominal). Confidence limits were not reported.

In addition, one result for *Lemna gibba* was obtained from the US EPA report. Exposure is assumed to be 14 days, and the EC50 and NOEC were reported as 0.695 and 0.058 mg/L respectively.

Conclusions for Aquatic Toxicity

Acute testing on fish showed a range of freshwater and marine fish species to be insensitive to 2,4-D in its acid form. The most sensitive species for which a defined end-point was obtained in acute testing was a 96 h LD50 of 175 mg/L for the marine tidewater silverside. A single acute amphibian test on leopard frog tadpoles resulted in a 96 h LC50 of 359 mg/L. One chronic study on fathead minnow showed a NOEC of 63.4 mg/L.

Aquatic invertebrates (water fleas, pink shrimp and eastern oysters) were variable in their sensitivity to 2,4-D. The most sensitive animal appeared to be *Daphnia magna* with a lowest 48 LC50 value of 25.0 mg/L. A single chronic test on daphnids gave a 21 day NOEC of 79 mg/L (corresponding EC50 of 235 mg/L).

Two tests on different green algae species showed relatively low toxicity of 2,4-D acid to algae with EC50s between 30-100 mg/L. However, the duckweed *Lemna gibba* appears much more sensitive, and 2,4-D is highly toxic with a reported EC50 of 0.695 mg/L from a study not assessed within this review.

Terrestrial Toxicity

Non-Target Invertebrates

Bees

One non-standard test was provided for toxicity of 2,4-D technical to honey bees.

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

Test system:

Toxicity of 2,4-D acid technical to worker bees (*Apis indica*), 5-15 days old, was tested using a dry film method. Thirteen treatments were performed with 2,4-D acid at 1, 2, 3, 4 and 5% w/v, endosulfan as a toxic standard at 0.006, 0.008, 0.011, 0.014, 0.017 and 0.020% v/v, a water control and a solvent (acetone) control. These concentrations were based on the results of pilot studies. Three replicates of each treatment were performed with 10 bees per replicate.

For pre-conditioning, bees collected from hives were kept in glass jars covered with muslin cloth. A cotton swab soaked in 50% sugar was placed inside for feed. The jar was kept at room temperature (around 24^{o}C) and relative humidity around 40-80% for 15 hours (of which 12 were in the dark).

For treatment, relevant concentrations of test substance were dissolved in acetone. 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 either the control or solvent 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 1, 2, 3, 4 and 5% w/v treatment groups after 24 hours (mean of three replicates) was 3.3, 13.3, 36.7, 33.3 and 40.0% respectively.

Conclusion:

Because less than 50% mortality was found at the highest treatment level, an LC50 could not be calculated. While 40% mortality was found at 5% 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

No data were provided for 2,4-D in its acid form.

Earthworms

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

Report: Kumar, 2001

Guidelines: OECD Guideline 207

GLP: No (Quality Assurance Statement provided).

Test system:

Toxicity of 2,4-D Acid technical to the earthworm, *Lampito mauritii* was tested using nine treatments, namely, control and 2,4 D at 100-800 mg/kg dry wt soil (increasing by 100 mg/kg at each treatment level). Four replicates were performed per treatment.

Doses were prepared in acetone. A known volume of spray solution for each treatment level was uniformly sprayed in each treatment tray (0.2 m^2 in area) containing 1 kg of soil mixed with farmyard manure (1:1). Before the start of the experiment, healthy worms of uniform size (6-7 weeks old) and weight (700-1000 mg/worm) were selected. 10 worms per replicate were used, and the worms were placed on the soil and allowed to acclimatise and burrow inside the soil. The plastic trays were covered with perforated plastic and kept under test conditions for 14 days.

Physiological impairments such as reduction in the weight and size along with mortality were observed after days 7 and 14. The test temperature was maintained at around 28^{o}C. The moisture content of the soil at pre- and post-treatment was estimated. The pH of the soil was determined.

Findings:

The pH of the soil before and after the experiment was 7.6 and 7.0 respectively. The moisture content remained the same at 85%.

No mortalities were found in the control group. The following table summarises the day 7 and 14 mortality and weight results:

Dose (mg/kg dw soil)	% Mortality		Average earthworm weight (g)		
	Day 7	Day 14	Pre-Treatment	Day 7	Day 14
Control	0	0	0.872	0.873	0.879
100	2.5	10.0	0.910	0.882	0.752
200	22.5	32.5	0.941	0.681	0.498
300	35.0	47.5	0.878	0.554	0.418
400	55.0	60.0	0.907	0.378	0.275
500	57.5	65.0	0.850	0.379	0.226
600	65.0	70.0	0.863	0.255	0.220
700	72.5	80.0	0.949	0.226	0.128
800	100	100	0.895	-	-

In terms of body weight change, at the end of the experiment, control worms had roughly maintained body weight (an average increase of around 1%). There was a very marked effect of 2,4-D exposure on weight of worms by comparison. At the 100, 200, 300, 400, 500, 600 and 700 mg/kg soil treatment groups, weight loss by day 14 was around 17, 47, 52, 70, 73, 75 and 87% respectively.

Conclusion:

The LD50, as calculated by probit analysis, was 327.2 mg/kg dw soil with confidence limits (assumed 95%) of 301.1-343.2 mg/kg dw soil.

Soil Micro-Organisms

No data were provided for 2,4-D in its acid form.

Non-Target Vegetation

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

Test system:

Tier II germination and seedling emergence studies were conducted over 14 days to evaluate non-target phytotoxicity of 2,4-D acid 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) 4260, 2130, 1065, 532, 270 and 135 g/ha for the *in vitro* Petri dish germination component and 4700, 2350, 1175, 594, 290, 146, 72.8, 33.6, 16.8 and 8.4 g ae/ha in the soil seedling emergence component. There were three replicates of each of the treatments, including the untreated controls.

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

For the seedling emergence in soil component of the test, the growth medium was steam-pasteurised natural soil amended with 50% silica sand and supplemental nutrients. The medium had a pH of 6-7, CEC around 4 meq/100 g and organic matter of <2.0%. 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 >90% of the nominal values for both the Petri dish and soil components of the study.

Seed Germination: Soybean and tomato were the most sensitive species in the germination Petri dish assay with 2,4-D acid generally showing only slight activity on seed germination of the other species. Although the statistical output for onion yielded a defined 4260 g/ha, the highest dose tested, for both the EC25 and EC50 values, inspection of the mean data for this species indicated that both these values were between 2130 and 4260 g/ha. Also, the statistically generated NOEL for soybean

was 270 g.ha. However, based upon raw germination percent data, it is probable the NOEL lies between 135 and 270 g/ha.

Visual Observations (emergence): Fourteen days after application to the seedling emergence soil test units, plants were harvested. Immediately prior to this, visual observations were made. All species in the solvent and untreated controls were observed to be unaffected. At the highest test rate, oats, corn, and tomato exhibited no symptoms. This rate was deemed the visual NOEL for oats and corn with neither species showing effects at lower levels. Mustard and radish exhibited necrosis, as did onion, which also exhibited stunting, and cucumber, which exhibited pronounced stunting. Stunting of varying degrees was observed on buckwheat, sorghum and soybean. Mustard and radish continued to exhibit necrosis at 2350 g ae/ha. Slight distortion was observed on tomato while onion evidenced stunting and distortion. Stunting was apparent on cucumber, sorghum and soybean with buckwheat appearing slightly stunted.

The visual NOEL for onion appeared to be 1175 g/ha; this species exhibited no symptoms at this rate or below. Stunting was observed on tomato and mustard with slight stunting on cucumber, sorghum and soybean. Radish continued to exhibit necrosis. No symptoms were observed on buckwheat. At the next rate, 594 g/ha and at all rates lower, cucumber and tomato showed no symptoms and this rate was deemed the visual NOEL for these species. At this rate, slight stunting was observed on buckwheat, sorghum and soybean. Necrosis was found on mustard and radish with radish also exhibiting stunting.

At 290 g/ha and below buckwheat and sorghum showed no symptoms and this level was the visual NOEL for these two species. Stunting was observed on mustard and radish with slight stunting on soybean. At 146 g/ha no effects were observed on soybean and this was the apparent visual NOEL for this species. Only mustard (stunting) and radish (stunting and necrosis) exhibited effects at this rate. At 72.8 g/ha and below, none of the species exhibited any observable effects. This rate appeared to be the visual NOEL for mustard and radish.

Seedling Emergence

Emergence

Mustard and radish were the most sensitive species of this phase of the test exhibiting NOELs of 1175 g ae/ha. Six of the species tested had NOELs of at least the highest tested level of 4700 g/ha.

Fresh weight

Tomato was the least sensitive species in this part of the test. Although the statistically generated NOELs for corn and oats were both higher than 4700 g/ha, significant growth reduction (16% and 17% respectively) occurred at this highest rate. The NOELs were re-assigned at 2350 g/ha.

While the values for cucumber were statistically appropriate, fit for the linear model was problematic and the raw data suggested the NOEL and EC25 for cucumber were lower than reported. Manual calculations of the NOEL indicate it was between 146 and 594 g/ha while the EC25 was considered to be between 594 and 1175 g/ha.

The statistically generated NOEL for radish was at best the lowest tested rate of 8.4 g/ha while the EC25 was 8.4 g/ha. Calculation of these values was based primarily on data at the lowest concentrations; the values were believed to be artificially low. The

statistical output was overridden and the NOEL for radish was reassigned at 16.8 g/ha with the EC25 predicted to lie between 33.6 and 72.8 g/ha.

Statistical Results

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

Table A1.40: Effects of 2,4-D Acid on Seedling Emergence and Germination of ten plant species (results in g/ha)

		Seed Germination			Seedling Emergence			Fresh Weight		
		NOEL	EC25	EC50	NOEL	EC25	EC50	NOEL	EC25	EC50
Monocots	Onion	2180	2130-4260	2130-4260	2354	4700	≥4700	2350	≥2350	3260
	Sorghum	1065	2154	≥4260	≥4700	≥4700	≥4700	2350	≥2350	≥4700
	Corn	2130	≥4260	≥4260	≥4700	≥4700	≥4700	2350	≥4700	≥4700
	Oats	532	1640[1]	≥4260	≥4700	≥4700	≥4700	2350	≥4700	≥4700
Dicots	Tomato	≤135	≤135	≤135	≥4700	≥4700	≥4700	≥4700	≥4700	≥4700
	Soybean	135-270	274[2]	1028[3]	2354	≥4700	≥4700	1175	1917	≥4700
	Buckwheat	≥4260	≥4260	≥4260	≥4700	≥4700	≥4700	1175	1445	4370
	Mustard	1065	1596	2914	1175	≥1175	3450	16.8	37	155
	Radish	2130	4260	≥4260	1175	1480	≥4700	16.8	33.6-72.8	291
	Cucumber	532	1320	2770	≥4700	≥4700	≥4700	146-594	594-1175	≥4700

Reported confidence limits in g ae/ha are: **1)** 955-4173; **2)** 78.5-504; **3)** 568-2251

Conclusions:

While percent emergence was less affected by the test chemical than germination, fresh weight data present a different situation. Although emergence appeared to be relatively high, statistical analysis of fresh weights of several species appeared to reveal chemical effects on the newly-emerged seedlings. For example, mustard and radish both showed emergence NOELs of 1175 g/ha, but the fresh weight NOELs were 16.8 g/ha.

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

Report: Backus, 1992b

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

GLP: No – QA statement provided.

Test system:

Tier II vegetative vigour studies were conducted to evaluate non-target phytotoxicity of 2,4-D acid to 6 dicotyledonous plant species and 4 monocotyledonous plant species. For corn, cucumber, soybean and tomato, several seeds per pot were planted to 1-1.5 cm depending upon the seed size. After emergence, thinning to one plant per pot was performed. Buckwheat, mustard, oats, onion, radish and sorghum were planted by sprinkling seed on the growing medium surface in the pot and covering with around 0.5-1 cm additional medium. Test material application occurred on a uniform population, thinned at seedling emergence to avoid crowding.

Plant species were within 7 to 21 days from planting when the test material was applied. Each treatment included three replicates. Each replicated contained 5 pots of corn, cucumber, soybean and tomato with each pot containing one plant. Each replicate for buckwheat, mustard, oats, onion, radish and sorghum consisted of 1 pot

each of a representative population. There were 3 solvent control replicates (acetone only) and 3 untreated control replicates.

Ten dose rates were tested equating in rounded values to 4700, 2350, 1175, 594, 290, 146, 72.8, 33.6, 16.8 and 8.4 g/ha. Test treatments were applied as a foliar spray using a moving laboratory sprayer.

Visual observations for survival and phytotoxicity were evaluated at 3, 7, 10 and 14 days after treatment. Following the day 14 observations, plants were harvested for the above ground portions and fresh weight analysed.

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

Findings:

Analytical verification of application concentrations showed >94% of the nominal concentration was found in spray mixes.

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

On day 14, at 4700 g/ha slight distortion was observed on corn. Buckwheat, cucumber and onion exhibited distortion and necrosis. Slight stunting and necrotic edges were observed on sorghum. Mustard, radish, soybean and tomato appeared necrotic. Oats did not exhibit any symptoms and this was the visual NOEL for this species. The 2350 g/ha rate appeared to be the visual NOEL for corn. At this rate, buckwheat and cucumber showed distortion while buckwheat exhibited necrosis and cucumber exhibited stunting. Necrosis was observed on mustard, onion, radish and tomato. Sorghum displayed slight stunting as well as necrotic edges. Soybean appeared distorted and necrotic.

The 1175 g/ha rate appeared to be the visual NOEL for sorghum. Distortion and stunting were found on buckwheat and cucumber. Distortion and necrosis were observed on mustard, onion, radish and soybean. Tomato appeared necrotic. Symptoms appeared less pronounced at the 594 g/ha rate. Buckwheat, cucumber, mustard, onion and radish all evidenced distortion while soybean displayed necrotic spots and tomato displayed necrosis and distortion. Symptoms were virtually identical for the 290 g/ha rate. Cucumber however, displayed stunting and necrosis on tomato was limited to spotting. Similar responses were found again at 146 g/ha although stunting was evident on onion only and no necrosis was observed on tomato.

The only symptom observed at the 72.8, 33.6 and 16.8 g/ha rates was distortion in varying degrees on the broadleaf species and onion. As the rates became lower, symptoms were less pronounced, with distortion at the 16.8 g/ha rate observed as "slight". At the lowest rate tested, 8.4 g/ha, only soybean and tomato displayed any symptoms. Distortion was noted on these species, only, at the lowest rate, however, it was slight.

Statistical results:

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

Table A1.41: Effects of 2,4-D Acid on vegetative vigour of ten plant species (results in g/ha)

		Fresh Weight		
		NOEL	EC25	EC50
Monocots	Onion	<8.4*	≤8.4	99.8[1]
	Corn	≥4700	≥4700	≥4700
	Oat	≥4700	≥4700	≥4700
	Sorghum	594*	381	3660
Dicots	Buckwheat	8.4	25.7[2]	204[3]
	Soybean	16.8*	9.0	43.7
	Cucumber	<8.4*	16.8	72.8
	Radish	8.4*	17.9[4]	130[5]
	Tomato	<8.4*	8.4	33.6
	Mustard	<8.4*	12.3	54.9

*Statistical output was overridden.
Reported confidence limits (g/ha) were **1)** 43.7-193; **2)** 13.5-40.4; **3)** 141-294; **4)** 13.2-23.5; and **5)** 109-156.

Monocots:

Onion (exhibiting a linear dose response) was the most sensitive monocot. The statistically generated NOEL and EC25 values were at best, the lowest rate tested of 8.4 g/ha. When onion raw mean fresh weight data were compared to the combined mean of the untreated and solvent controls, at the lowest rate, 33% inhibition was found. At this lowest rate, onion response was 80% of the solvent control. Despite the solvent controls not being significantly different, comparison with the solvent control only indicates significant effects at the two lower concentrations. The statistically generated NOEL for sorghum was 1175 g/ha, however, the mean fresh weight data at this level indicated a response equal to 83.7% of the combined control. Given that response at the next lowest level, 594 g/ha, was approximately equal to that of the combined control and all rates below this evidenced a response greater than the combined control responses, the NOEL was reassigned a value of 594 g/ha.

Dicots

All broadleaf species exhibited sensitivity to the test material. Cucumber, mustard and tomato were the most sensitive dicots in this test. Compared with the pooled control at 8.4 g/ha, cucumber mean fresh weight was 85.1%, mustard was 81.6% and tomato was 72.4%. Because cucumber demonstrated low variance, inhibition at 8.4 g/ha appeared to be significant. Consequently, the statistically derived NOELs for these species were overridden meaning a NOEL could not be defined as the lowest tested level was 8.4 g/ha.

The response of radish at 8.4 g/ha was 93% that of the control and this appeared to be the NOEL for this species. Although soybean response was 20% and 30% reduced at the two lowest concentrations when compared to the solvent control, growth at these two concentrations was >90% of the untreated control, hence the NOEL for this species was amended upwards from the statistically derived NOEL to 16.8 g/ha.

Conclusions:

Dicots were much more sensitive to monocots when exposed to 2,4-D Acid in a vegetative vigour study with the exception of onion, a monocot, being the most sensitive species tested. Cucumber, mustard and tomato were the most sensitive dicots based on manually derived NOELs with tomato being the most sensitive based on EC25s with a value of 8.4 g/ha, the lowest level tested.

Conclusions for Terrestrial Toxicity

No standard toxicity tests were performed on terrestrial organisms using 2,4-D in its acid form. This is acceptable as tests were performed with 2,4-D in various ester or salt forms that are used in formulations, and therefore are of more relevance. Some data are available for 2,4-D in its acid form to non-target vegetation through seedling emergence and vegetative vigour studies. The results of these data indicate that 2,4-D acid is much more toxic to dicotyledons than monocotyledons, the exception being onion, a monocot, that was the most sensitive species to 2,4-D acid in the vegetative vigour study. Secondly, emerged plants appeared to be more sensitive than pre-emerged plants with impacts on seed germination and percent emergence being relatively slight compared to impacts on dicotyledon fresh weights following foliar application to emerged plants.

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