Data Evaluation Record on Propanil Metabolite Hydrolysis under Laboratory Conditions
Propanil & Metabolite: PC 028201&600166, EPA MRID Number: 47165601, DP Barcode: 343055

Test Material:	3,4-Dichloroaniline (transformation product of propanil)
MRID	47165601
Title:	Penketh, S. 2004. Propanil metabolite hydrolysis under laboratory conditions.
EPA PC Code:	028201 (for propanil)
OCSPP Guideline:	835.2120

For Cambridge Environmental

Primary Reviewer: Kindra Bozicevich **Signature:** 

Date: 1/19/12

Secondary Reviewer: Kathleen Ferguson **Signature:** 

Date: 1/19/12

QC/QA Manager: Joan Gaidos **Signature:** 

Date: 1/19/12

IIA 7.5. Hydrolysis of 3,4-dichloroaniline, a transformation product of propanil, at pH 4, 7, and 9

The hydrolysis of 3,4-dichloroaniline was studied in aqueous buffer solutions at pH 4, 7, and 9 at 50°C for up to 5 days.

Report:	Penketh, S. 2004. Propanil metabolite. Hydrolysis under laboratory conditions. Unpublished study performed by Huntingdon Life Sciences, Ltd., Cambridgeshire, England; sponsored and submitted by Propanil Consortium, Washington, DC. Huntingdon Life Sciences Project ID: JSV/015. Experiment started May 16, 2003, and completed August 28, 2003. Final report issued July 27, 2004.
Document No.:	MRID 47165601
Guideline:	Conducted under: OECD No. 111; EEC Council Directive 91/414/EEC/ SETAC Europe Reviewed under: OCSPP 835.2120
Statements:	The study was conducted in accordance with UK and OECD GLP standards. Signed and dated Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-3, 40). A Certification of Authenticity statement was not provided.
Classification:	This study is classified as acceptable. No significant deviations from good scientific practices or OCSPP guidelines were noted.
PC Code:	600166 (PC Code for propanil 028201)

Reviewer:	He Zhong, Ph.D. Biologist Environmental Risk Branch I	**Signature:** **Date:** February 29, 2012

Executive Summary

The abiotic hydrolysis of [phenyl-U-^{14}C]-labeled 3,4-dichloroaniline, at 10.40-10.57 mg a.i./L, was investigated in 0.01M sterile aqueous buffered solutions at pH 4 (acetate), 7 (phosphate), and 9 (borate). The test was conducted in the dark at 50 ± 2°C for 5 days. As a result of significant changes in the pH values of test solutions in the 0.01M pH 9 experiment, the experiment was repeated at pH 9 using a 0.1M buffer solution. Duplicate samples were collected and analyzed using LSC, HPLC, and one- and two-dimensional TLC. GC/MS was used to confirm the presence of 3,4-DCA in the pH 9 buffer solutions.

It was noted that the pH of the pH 4 and pH 7 test solutions decreased by *ca.* 0.2 and 1 unit, respectively, during the 5 day experiment. The pH of the 0.01M pH 9 solution decreased by *ca.* 3.1 units, and the pH 0.1M pH 9 solution by *ca*. 2.8 units.

Table 1. Results Synopsis.

pH	Half-life	Transformation products (Common name (maximum %AR [A] observed, associated interval))	
		Major	Minor Identified
4	Stable.	None	None
7	Stable		
9	Stable.		

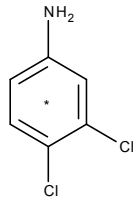
[A] AR means "applied radioactivity".

I. Material and Methods

A. Materials:

1. Test Material [Phenyl-U-^{14}C]3,4-dichloroaniline (3,4-DCA; p. 12).

Phenyl-U-^{14}C



Specific activity: 20.8 mCi/mmol; 4.75 MBq/mg

Radiochemical purity: >97% (HPLC, TLC; p. 19; Appendix 1, p. 35)

Analytical purity: Not reported.

Batch No.: NPE/HLS247/3

Solubility in water: 580 mg/L at 20°C.

2. Reference compounds:

3,4-Dichloroaniline Batch No. HBO11667, purity 98.8% (p. 13)

3. Buffer:

Table 2. Buffer solutions.

pH	Type and molarity of the buffer	Composition
4	0.01M Acetate	Glacial acetic acid (0.3 mL) was diluted in water (*ca.* 450 mL), the pH was adjusted with aqueous NaOH, and the volume brought to 500 mL.
7	0.01M Phosphate	Sodium dihydrogen orthophosphate (0.78 g) was dissolved in water (*ca.* 450 mL), the pH was adjusted with aqueous NaOH, and the volume brought to 500 mL.
9	0.01M Borate	Boric acid (0.309 g) was dissolved in water (*ca.* 450 mL), the pH was adjusted with aqueous NaOH, and the volume brought to 500 mL.
9	0.1M Borate	Boric acid (3.09 g) was dissolved in water (*ca.* 450 mL), the pH was adjusted with aqueous NaOH, and the volume brought to 500 mL.

Data obtained from pp. 13-14 in the study report.

B. Study Design

1. Experimental conditions: The abiotic hydrolysis of [phenyl-U-^{14}C]-labeled 3,4-DCA at measured concentrations ranging from 10.40-10.57 mg/L was investigated in sterile aqueous buffered solutions at pH 4, 7, and 9 (p. 13; Appendix 3, p. 37). Equipment was sterilized by autoclaving (p. 14). The test was conducted at a nominal concentration of 10 mg a.i./L in sealed borosilicate glass tubes maintained in the dark at 50 ± 2°C for 5 days (p. 19). Volatiles were not collected. The cosolvent used was ethyl acetate and was <1% of the sample solutions. Measured concentrations were 10.57, 10.43, and 10.40 mg/L for the pH 4, 7, and 9 buffer solutions, respectively. As a result of significant changes in the pH values of test solutions in the 0.01M pH 9 experiment, a repeat experiment using 0.1M pH 9 buffer solution was conducted at a measured concentration of 10.43 mg/L. The sterility of the test solutions was not confirmed.

2. Sampling: Duplicate samples (0.25 mL) were collected for analysis after 0, 2 and 5 days of incubation (p. 15). The pH of the test solutions was checked at each sampling interval.

3. Analytical procedures: Samples were analyzed using LSC for determination of total radioactivity (p. 15). HPLC (Solvent A: 0.1% phosphoric acid in acetonitrile; Solvent B: 0.1% phosphoric acid in water) with UV (254 nm) and radiodetection and one-and two dimensional normal phase TLC [chloroform:methanol (9:1, v:v) and chloroform:methanol:ammonia (90:10:1, v:v:v)] were used for the separation and quantitation of products formed (pp. 16-17).

In addition, the Day 5 0.1M pH 9 solution was analyzed using GC-MS with electron impact ionization (EI) to confirm the presence of 3,4-DCA in the pH 9 buffer solutions (pp. 17-18).

Limits of Detection (LOD) and Quantitation (LOQ) were not reported.

II. Results and Discussion

A. Mass Balance: Recoveries ranged from 97.2-99.4% of the applied in the pH 4 buffer, 88.1-99.6% in the pH 7 buffer, 87.7-99.9% in the 0.01M pH 9 buffer, and 94.4-100.2% in the 0.1M pH 9 buffer (Tables 1-3, pp. 22-24). There was no significant loss of radioactivity from the test systems over time.

B. Findings: The results including total recovery and distribution of radioactivity are presented in **Tables 3a-3d**.

Table 3a. Hydrolysis of 3,4-DCA at pH 4 at 50°C, expressed as a percentage of the applied radioactivity.

pH 4, 50°C			
	Sampling Interval (days)		
Component	**0**	**2**	**5**
3,4-DCA	99.2	99.3	99.3
Transformation products[1]	0.2	ND	ND
$^{14}CO_2$ and other volatiles	Volatiles were not trapped.		
Total recovery	99.3, 99.4	97.9, 97.2	98.2, 98.1

Data were obtained from Table 1, p. 22 and Table 4, p. 25 of the study report. Two samples were analyzed at each sampling interval, but only mean concentrations of 3,4-DCA were reported by the study author.
1 Calculated by the reviewer as the difference between the mean concentration of 3,4-DCA and total recovery.

Table 3b. Hydrolysis of 3,4-DCA at pH 7 at 50°C, expressed as a percentage of the applied radioactivity.

pH 7, 50°C			
	Sampling Interval (days)		
Component	**0**	**2**	**5**
3,4-DCA	98.2	99.3	99.2
Transformation products[1]	1.4	ND	ND
$^{14}CO_2$ and other volatiles	Volatiles were not trapped.		
Total recovery	99.6, 99.5	88.1, 92.3	96.5, 97.1

Data were obtained from Table 2, p. 23 and Table 4, p. 25 of the study report. Two samples were analyzed at each sampling interval, but only mean concentrations of 3,4-DCA were reported by the study author.
1 Calculated by the reviewer as the difference between the mean concentration of 3,4-DCA and total recovery.

Table 3c. Hydrolysis of 3,4-DCA at 0.01M pH 9 at 50°C, expressed as a percentage of the applied radioactivity.

pH 9 (0.01M), 50°C			
	Sampling Interval (days)		
Component	**0**	**2**	**5**
3,4-DCA	99.2	99.7	99.7
Transformation products[1]	0.6	ND	ND
$^{14}CO_2$ and other volatiles	Volatiles were not trapped.		
Total recovery	99.6, 99.9	98.4, 98.3	87.7, 98.1

Data were obtained from Table 3a, p. 24 and Table 4, p. 25 of the study report. Two samples were analyzed at each sampling interval, but only mean concentrations of 3,4-DCA were reported by the study author.
1 Calculated by the reviewer as the difference between the mean concentration of 3,4-DCA and total recovery.

Table 3d. Hydrolysis of 3,4-DCA at 0.1M pH 9 at 50°C, expressed as a percentage of the applied radioactivity.

pH 9 (0.1M), 50°C			
	Sampling Interval (days)		
Component	**0**	**2**	**5**
3,4-DCA	---	---	98.7
Transformation products[1]	---	---	2.7
$^{14}CO_2$ and other volatiles	Volatiles were not trapped.		
Volatiles	99.8, 100.2	99.0, 98.7	97.5, 94.4

Data were obtained from Table b3, p. 24 and Table 4, p. 25 of the study report. Samples were analyzed for 3,4-DCA only at 5 days posttreatment. Two samples were analyzed by LSC at each sampling interval, but only mean concentrations of 3,4-DCA were reported by the study author.
1 Calculated by the reviewer as the difference between the mean concentration of 3,4-DCA and total recovery.
--- = not analyzed

Table 4. Hydrolysis kinetics of [^{14}C]3,4-DCA in aqueous buffer solutions.

Temp./pH		50°C
4	Linear SFO Half-life	Stable.
	Observed DT_{50}	>5 days
	Observed DT_{90}	>5 days
7	Linear SFO Half-life	Stable.
	Observed DT_{50}	>5 days
	Observed DT_{90}	>5 days
9	Linear SFO Half-life	Stable.
	Observed DT_{50}	>5 days
	Observed DT_{90}	>5 days

[Phenyl-U-^{14}C]3,4-DCA was stable in pH 4, 7, and 9 buffer solutions at 50°C accounting for maximums of 98.2-99.2%AR at 0 days and 98.7-99.7% after 5 days (Table 4, p. 25).

No major transformation products were isolated, and minor transformation products were not individually quantified or identified.

At pH 4 and 7, the pH of the test decreased from 4.11 to 3.89 and from 7.07-7.12 to 6.13-6.16, respectively, during the 5 day study (p. 19; Tables 1-2, pp. 22-23). In the pH 9 0.01M solution, the pH of the test solutions declined from 8.71-8.74 at 0 days to 5.55-5.56 after 5 days (Table 3a, p. 24). In a repeat experiment using a pH 9 0.1M buffer solution, the pH of the test solutions declined from 9.00-9.01 at 0 days to 6.17-6.19 after 5 days (Table 3b, p. 24). GC-MS analysis confirmed that 3,4-DCA was the only compound present in the 0.1M pH 9 test solutions at 5 days posttreatment (p. 20).

III. Study Deficiencies and Reviewer's Comments

1. The pH of all of the buffer solutions decreased during the 5 day experiment (Tables 1-3, pp. 22-24). The most significant decline (*ca.* 3 units) occurred in the pH 9 buffer solutions, and increasing the buffing capacity of the solution 10x had minimal additional buffering effect. The study author did demonstrate that 3,4-DCA was stable in the pH 9 solutions after 5 days.

2. Material balances were *ca.* 88% of the applied in single samples of the pH 7 and 9 buffer solutions (Tables 2-3, pp. 23-24). In both cases, replicate samples were >90% of the applied

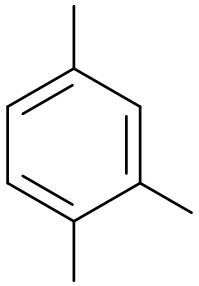
3. Limits of Detection and Quantitation were not reported.

4. The sterility of the test solutions was not confirmed. However, this did not impact interpretation of the study results since 3,4-DCA was stable in all solutions.

5. The study author only provided averaged concentrations for 3,4-DCA (Table 4, p. 25). It is preferred that data from individual samples be reported so that between sample variability can be assessed.

6. The solubility of 3,4-DCA at 10 mg/L was confirmed prior to the definitive experiment (p. 14). Mean recoveries from solution ranged from 99.7-101.5% (p. 19; Appendix 2, p. 36).

DER ATTACHMENT 1. 3,4-DCA (Transformation Product of Propanil). [A]

Code Name/ Synonym	Chemical Name	Chemical Structure	Study Type	MRID	Maximum %AR (day)	Final %AR (study length)
PARENT						
3,4-DCA	3,4-Dichloroaniline **Formula:** $C_6H_5C1_2N$ **MW:** 162.02 g/mol **SMILES:** c1cc(c(cc1N)Cl)Cl		835.2120 Hydrolysis	47165601	99.3% (2, 5 d; pH 4)	99.3% (5 d; pH 4)
					99.3% (2 d; pH 7)	99.2% (5 d; pH 7)
					99.7% (2, 5 d; 0.01M pH 9)	99.7% (5 d; 0.01M pH 9)
					98.7% (5 d; 0.1M pH 9)	98.7% (5 d; 0.1M pH 9)
MAJOR (>10%) TRANSFORMATION PRODUCTS						
No major transformation products were identified.						
MINOR (<10%) TRANSFORMATION PRODUCTS						
No minor transformation products were identified.						
REFERENCE COMPOUNDS NOT IDENTIFIED						
All compounds used as reference compounds were identified.						

[A] AR means “applied radioactivity”. MW means “molecular weight”. NA means "not applicable".

Attachment 2: Statistics Spreadsheets and Graphs (N/A)