RESPONDENT’S EXHIBIT 12

Aerobic degradation of DCPA (Dacthal) in aquatic systems

Report:	MRID 49307515. Nelson, T.R. 1984. (p. 12). An Aerobic Aquatic Soil Metabolism Study with 14C-Dacthal (Revised per PRN 11-03 and 86-5). Unpublished study performed by SDA Biotech Corporation, Painesville, Ohio; sponsor unknown; submitted by AMVAC Chemical Company, Newport Beach, California (pp. 1, 12). SDS Study No.: 699-3EF-84-0073-001. AMVAC ID: 100-MET-010a. Experiment started July 16, 1984, and terminated November 1, 1984 (p. 12). Final report issued January 29, 2014.
Document No.:	MRID 49307515
Guideline:	OCSPP 835.4300
Statements:	The study was conducted according to GLP standards (p. 6). Signed and dated Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2, 6, 27). The authenticity of the report is certified in the Quality Assurance statement (p. 27).
Classification:	This study is supplemental. No time 0 samples were collected. Soil properties such as pH and organic carbon content were not reported, and the soil was oversieved (600 µm) prior to use. No information was provided on the redox potentials in the water and soil before and during the experiment.
PC Code:	078701
Reviewer:	

James Lin, Environmental Engineer
U.S. EPA

Signature:
Date: January 19, 2017

Executive Summary

The aerobic transformation of [phenyl-U-^{14}C]DCPA (dacthal, SDS-893) was studied in a water:silty clay loam soil system (water pH 8.6, soil uncharacterized) from Ohio in closed systems treated at 0.43 mg a.i./L and incubated in the dark at 25°C for up to 90 days. Samples were opened to the air for at least 1 hour/working day. Duplicate samples (two entire bottles) were collected at each sampling interval. Redox potentials, oxygen saturation and pH of the test system were not reported. It was not demonstrated that the test system was viable before or during the study.

Overall mass balances averaged 100.8 ± 4.6% (range 92.1-110.7%) of the applied. Recoveries were variable but generally within guideline criteria (90% - 110%). In the water column, DCPA decreased from a maximum of 32.2% of the applied at 4 days (first sampling interval) to 1.2% at 90 days posttreatment. In the soil, DCPA was 72.8% at 4 days, a maximum 77.9% at 14 days, and 27.1% at 90 days.

The observed DT$_{50}$ value, calculated half-life, and information on transformation products are listed in **Table 1**. DCPA dissipated with a SFO DT50 of 45.5 days. Two transformation products were identified.

No time 0 samples were collected. In the water, total radioactive residues were 39.4% at 4 days posttreatment and increased to a maximum 80.4% at 90 days. In the soil, extractable radioactivity was 77.2% at 4 days, increased to a maximum of 83.8% at 14 days, and was 30.8% at 90 days. Unextracted radioactivity was ≤1.4% in all samples except for one replicate at 7 days (3.4%). Volatiles were not measured.

Table 1. Results Synopsis: Aerobic Aquatic Metabolism of DCPA in the Total System[1]

Total System	Observed DT_{50} (days)	Calculated Half-life (days)[1]	Model Parameters and Statistics[1]	Transformation Products Common Name (maximum % AR, associated interval)[2]	
				Major	**Minor**
Ohio, USA Water:silty clay loam soil (25°C, water pH 8.6, soil pH not reported)	33-46	45.5 SFO	$C_0 = 105$ $k = 0.0152$ $S_C = 863$ $S_{SFO} = 911$	MTP (54.9%, 90 days) TPA (26.2%, 90 days)	None

1 Calculated half-lives and model parameters in accordance with NAFTA kinetics guidance (USEPA, 2012); Single First Order (SFO).
2 AR means “applied radioactivity”.
MTP = Monomethyl tetrachloroterephthalate, DCPA monoacid, SDS-1449.
TPA = Tetrachloroterephthalic acid, DCPA diacid, SDS-954.

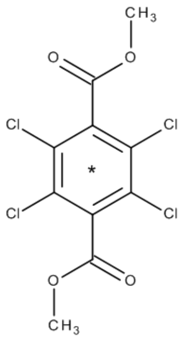
I. Materials And Methods

A. Materials:

1. Test Material [Phenyl-U-^{14}C]DCPA (p. 13).

Specific activity: 167,170 dpm/µg
Radiochemical purity: 98.5%
Chemical purity: Not reported

Lot No.: 719476



Solubility in water: Not reported

2. Reference Compounds: The following standards were used in the analysis (**Table 2**).

Table 2. Reference Compounds.

Applicant’s Code Name	Chemical Name	Purity (%)	Batch No.
SDS-893; Dacthal; DCPA	Dimethyl tetrachloroterephthalate; Dimethyl 2,3,5,6-tetrachlorbenzene-1,4-dicarboxylate	--	--
SDS-954; Dacthal diacid	Tetrachloroterephthalic acid, TPA	--	--
SDS-1449; Dacthal monoacid	Monomethyl tetrachloroterephthalate, MTP	--	--

Reference compounds identified in Figure 1, p. 19, in the study report. IUPAC names from the open literature.
-- = Not reported.

3. Water:Soil: Water and soil collection and characterization are summarized in **Table 3** and **Tables 4a-4b**, respectively.

Table 3. Water:Soil Collection and Storage.

Description		Details
Geographic location		Painesville, Ohio. Water from the Grand River, soil not specified.
Site description		Not reported
Pesticide use history at the collection site		Not reported
Collection date		Not reported
Collection procedures	Water:	Not reported
	Soil:	
Storage conditions	Water:	Not reported
	Soil:	
Storage length		Not reported
Preparation	Water:	Not reported
	Soil:	Ground with a mortar and pestle, then sieved (600 µm mesh).

Data obtained from pp. 13, 15, in the study report.

Table 4. Parameters for Characterization of Water:Soil Samples.

Parameter (unit)		Field Sampling/Post Handling	Stage of Test Procedure		
			Day 0	Day 46	Day 90
Water					
Temperature (ºC)		--			
pH		8.6	--	--	--
Hardness (mg/L as $CaCO_3$)		204			
TOC (µg/g)		--	--		--
O_2 Content (% saturation)		--	--	--	--
Measured redox potential (mV)			--	--	--
Soil					
Sampling Depth		--			
pH	1:2 soil:water	--	--	--	--
Soil Texture (USDA)		Silty clay loam			
Particle Size Distribution (%)	Sand	--			
	Silt	--			
	Clay	--			
Organic matter (%)		--			
Organic carbon (%)		--			
CEC (meq/100 g)		--			
Microbial biomass (µg C/g soil)		--	--		
Redox potential (mV)			--	--	--

Data obtained from pp. 13, 43 in the study report.
-- = not reported

B. Study Design

1. Experimental Conditions: Table 5 summarizes the experimental conditions.

Table 5. Experimental Design.

Experimental Design	Old Basing
Duration of the test	90 days

Experimental Design	**Old Basing**			
Water:				
Type and size of filter used	Not reported			
Amount of soil and water per treatment:				
Water (mL)	50 mL			
Soil (g dry wt)	25 g			
Water:soil ratio	2:1, w:w			
Application rates:				
Nominal	Not reported			
Actual	0.425 mg/L			
Number of replicates:				
Control, if used	Sterile controls were not used.			
Treated	Duplicate samples (two entire bottles) were collected at each sampling interval.			
Test apparatus:				
Type/material/volume	Amber bottles (4-oz volume) containing treated water and soil were loosely capped and incubated in darkness in an environmental chamber. For at least 1 hour each working day, the caps were removed to encourage aerobicity.			
Details of traps for CO_2 and organic volatile, if any	Volatiles were not trapped.			
If no traps were used, is the system closed?	Closed, except for ≥1 hour each working day.			
Identity and final concentration (based on water volume) of co-solvent	None			
Test material application method:				
Volume of the test solution used/treatment	50 mL			
Application method (*i.e.*, mixed/not mixed)	2 L of water plus 1.18 mg of test substance were mixed with constant stirring for 96 hours. The solution was then filtered, and aliquots (50 mL) were added to the bottles containing soil.			
Any indication of the test material adsorbing to the walls of the test apparatus?	None			
Microbial biomass (mg C/g)	Initial	Final	Initial	Final
Water	Not reported			
Soil				
Experimental conditions:				
Temperature	25 ± 1°C, range not provided			
Continuous darkness (yes/no)	Yes			
Other details (if any)	None			

Data obtained from pp. 14-15, of the study report.

2. Sampling during Study Period: Table 6 summarizes sampling during the study period.

Table 6. Sampling during Study Period.

Parameter	**Details**
Sampling intervals	4, 7, 14, 33, 46, 60, and 90 days posttreatment.
Sampling method	Duplicate samples (two entire bottles) were collected at each sampling interval.
Method of collection of CO_2 and organic volatile compounds	Volatiles were not addressed.
Sampling Intervals/Times	
Redox potential in water layer	Not reported

Parameter	Details
Dissolved oxygen in water layer	
pH in water layer	
Redox potential in soil	
pH in soil	
Other details, if any	None. Storage conditions and length of storage prior to analysis were not reported.

Data obtained from p. 15 of the study report.

3. Analytical Procedures:

Separation of the Water and Soil: The bottle containing the water and soil was agitated, then the mixture was filtered through a fritted glass filter funnel (p. 16). Aliquots of the water were analyzed using LSC.

Extraction/Clean Up/Concentration Methods: The water was acidified with concentrated HCl, then partitioned twice against diethyl ether (p. 16). Aliquots of the resulting organosoluble and aqueous fractions were analyzed using LSC.

The soil was extracted twice with acetone:0.3N HCl (80:20, v:v) then washed with acetone by filtering the solvent through the soil while it was still in the funnel (p. 17). Aliquots of the combined extracts were analyzed using LSC. The acetone was removed from the remaining extract under vacuum, and the resulting aqueous phase was partitioned twice against diethyl ether. Aliquots of the resulting organosoluble and aqueous fractions were analyzed using LSC.

The organosoluble fractions from the water and soil were concentrated using a rotovap, then diluted with acetonitrile and analyzed using HPLC.

Determination of Unextracted Residues: The extracted soils were dried, ground and analyzed for total radioactivity by LSC following combustion (p. 18).

Determination of Volatile Residues: Volatiles were not addressed.

Total Radioactivity Measurement: Total [^{14}C] residues were determined by summing the concentrations of residues measured in the water, soil extracts, and extracted soil (Table 1, p. 20).

Identification and Quantification of Parent and Transformation Compounds: Aliquots of the water and soil extracts were analyzed by HPLC using a column containing ODS-2 and eluted with a gradient mobile phase of A) acetonitrile and B) water (p. 17). Fractions were collected and analyzed using LSC. HPLC peaks were identified by comparison to reference standards that were cochromatographed with the samples.

Detection Limits (LOD, LOQ) for the Parent and Transformation Products: Limits of Detection and Quantification were not reported.

II. Results and Discussion

A. Data:

Study results, including total mass balances and distribution of radioactivity, are presented in **Table 7**. Redox potentials, oxygen saturation and pH were not reported for the water or the soil. The viability of the test system was not determined.

B. Mass Balance:

Overall mass balances averaged 100.8 ± 4.6% (range 92.1-110.7%) of the applied (Table 1, p. 20). Recoveries were variable but generally within guideline criteria (90% - 110%). No samples were collected at time 0. In the water column, DCPA decreased from a maximum of 32.2% of the applied at 4 days to 1.2% at 90 days posttreatment (Table 2, pp. 21-22). In the soil, DCPA was 72.8% at 4 days, a maximum 77.9% at 14 days, and 27.1% at 90 days.

C. Bound and Extractable Residues:

In the water, radioactive residues were 39.4% at 4 days posttreatment (first sampling interval) and increased to a maximum 80.4% at 90 days (Table 1, p. 20). In the soil, extractable radioactivity was 77.2% at 4 days, increased to a maximum of 83.8% at 14 days, and was 30.8% at 90 days. Unextracted radioactivity was ≤1.4% in all samples except for one replicate at 7 days (3.4%).

D. Volatilization:

Volatiles were not measured.

Table 7. Aerobic transformation of [^{14}C]DCPA, expressed as a percentage of applied radioactivity, in water:silty clay loam sediment.

Sampling Interval (days)	**4**		**7**		**14**		**33**		**46**		**60**		**90**	
Replicate	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**
DCPA (SDS-893)	94.3	94.8	90.5	92.4	89.4	89.4	79.7	80.1	50.7	40.9	37.2	41.9	28.3	15.5
Dacthal diacid (SDS-954)	0.3	0.4	0.4	0.5	0.6	0.9	2.4	2.0	8.1	12.9	16.4	13.3	19.5	26.2
Dacthal monoacid (SDS-1449)	1.9	1.7	3.0	3.1	5.3	6.0	14.5	14.3	38.1	43.3	43.1	41.1	46.6	54.9
Polar compounds	0.3	0.3	0.1	0.1	0.1	0.1	0.3	0.3	0.2	0.2	0.2	0.2	0.2	0.2
Water	25.3	39.4	22.2	26.2	17.6	24.4	32.1	32.2	50.8	64.2	59.1	55.1	69.9	80.4
Extracted residues	77.2	70.3	72.6	71.6	83.8	67.2	68.7	66.3	50.7	40.9	37.7	38.1	30.8	19.9
Unextracted residues	0.8	1.0	3.4	1.2	1.0	0.5	1.0	1.4	1.4	0.7	1.3	1.2	1.2	1.0
CO$_2$	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Organic Volatiles	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Mass balance	103.3	110.7	98.2	99.0	102.4	92.1	101.8	99.9	102.9	105.8	98.1	94.4	101.9	101.3

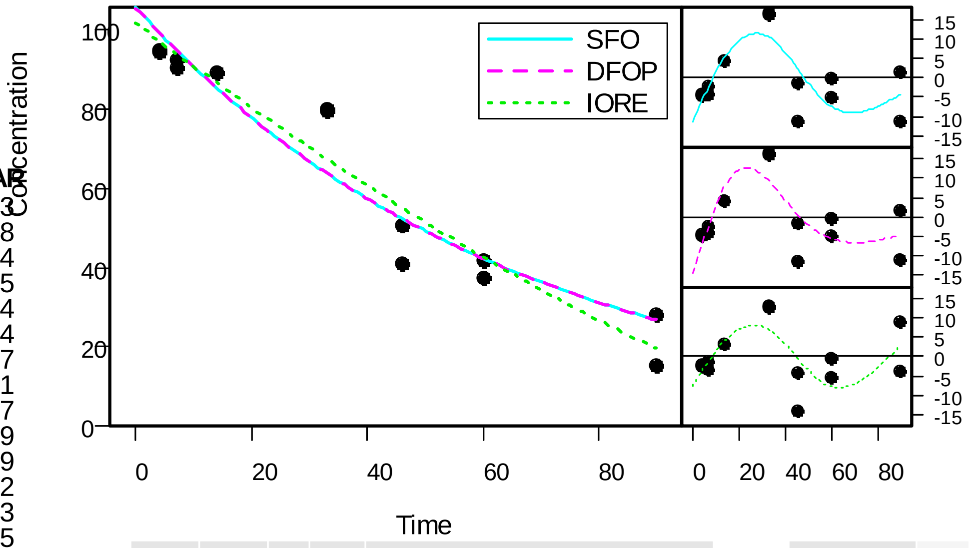
Data obtained from Tables 1-2, pp. 20-22, of the study report. Unextracted residue values from Table 1.
No time 0 samples were collected.
n.a. = not analyzed.
SDS-954 = TPA; Tetrachloroterephthalic acid, DCPA diacid.
SDS-1449 = MTP; Monomethyl tetrachloroterephthalate, DCPA monoacid.

E. Transformation of Parent Compound: Transformation kinetics of the parent compound in the total system based on individual sample data is summarized in the following **Figure**, with transformation product information summarized in **Table 8**.

Using a least squares linear regression program (not identified) and averaged data for each interval, the study author determined a total system half-life of 39 days (p. 24; Figure 2, p. 25).

DCPA in aerobic water:silty clay loam sediment

Da	%AR
4	94.3
4	94.8
7	92.4
7	90.5
14	89.4
14	89.4
33	79.7
33	80.1
46	50.7
46	40.9
60	41.9
60	37.2
90	28.3
90	15.5



	DT$_{50}$	DT$_{90}$	$\square^2$	C$_0$	Parameters
SF	45.	15	8.	10	k □0.0152
DF	45.	15	1	10	f □0.558□k$_0$ □0.0152□k$_1$ □0.0152
IOR	50.	10	8.	10	N □0.29□k □0.287

S$_C$	863
S$_{SFO}$	911
Slow□t$_{1/2}$	45.5
t$_{R□IORE}$	31.7

Kinetics models: Single First Order (SFO); Double First Order in Parallel (DFOP), and Indeterminate Order Rate Equation (IORE) in accordance with NAFTA kinetics guidance (USEPA, 2012).

Table 8. Transformation Products of DCPA in Aerobic Water:soil systems.

	Transformation Products	Maximum %AR Observed	Associated Interval (days)	Final %AR Observed	Final Interval (days)
Ohio, USA Water:silty clay loam soil (25°C, water pH 8.6, soil pH not reported)	MTP	54.9	90	54.9	90
	TPA	26.2	90	26.2	90

Data obtained from Table 2, pp. 21-22, in the study report.
MTP = Monomethyl tetrachloroterephthalate, DCPA monoacid, SDS-1449.
TPA = Tetrachloroterephthalic acid, DCPA diacid, SDS-954.

A transformation pathway was not provided by the study author. DCPA degraded to MTP which is turn degrades to TPA. Relatively little is adsorbed to the soil, and based on the material balances little or none appears to be is converted to CO$_2$ or organic volatile compounds (Table 1, p. 20).

III. Study Deficiencies and Reviewer's Comments

1. No time 0 samples were collected. The study author assumed 100% at time 0 for the half-life calculations (Figure 2, p. 25).

2. The soil was described as a silty clay loam from Painesville, Ohio (p. 13). No other information was provided. It was not specified whether the “soil” was a sediment and whether it was co-located with the water. The text refers to the soil properties being presented in Appendix II, but there is no Appendix II (p. 15). Based on the page numbers the document is complete and no pages are missing (i.e., the last page is Page 44 of 44). Soil collection dates and procedures were not reported, and it was not demonstrated that the soil was pesticide-free prior to use.

3. The soil was ground and sieved through a 600 µm mesh sieve (p. 15). A 2-mm mesh sieve is used in standard practice, since the finer mesh would remove much of the sand fraction of the soil.

4. No information was provided on the redox potentials in the water and soil before and during the experiment. The dissolved oxygen concentrations in the water were not reported. The pH of the water was only reported prior to treatment, and the pH of the soil was not reported. It was not known if the test system was oxic or suboxic during the study. The only method used to aerate the system was to open the sample bottles for at least an hour on each working day (p. 15).

5. The viability of the test systems was not determined before or at any time during the experiment.

6. Storage conditions and length of storage prior to analysis were not reported.

7. A single test system was used in the study. Aerobic aquatic metabolism data should be provided for at least two different water:sediment systems.

8. Limits of Detection and Quantification were not reported.

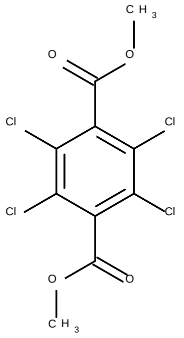
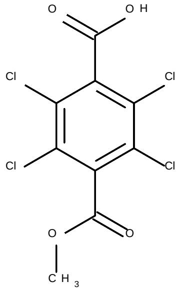
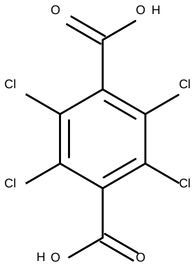
9. In a supplementary experiment, the dissipation of DCPA in the absence of soil under study conditions was evaluated (p. 15). DCPA was relatively stable in the water, with 92.0% of the applied DCPA undegraded at 91 days posttreatment (Table 4, p. 24). At 91 days, TPA comprised 0.5% of the applied and MTP comprised 4.3%. The study author concluded that the metabolism observed in the definitive experiment was occurring in the soil layer.

IV. References

1. U.S. Environmental Protection Agency. 2008. Fate, Transport and Transformation Test - Guidelines, OPPTS 835.4300, Aerobic Aquatic Metabolism. Office of Prevention, Pesticides and Toxic Substances, Washington, DC. EPA 712-C-08-018.

2. U.S. Environmental Protection Agency. 2012. NAFTA Guidance for Evaluating and Calculating Degradation Kinetics in Environmental Media.

DER ATTACHMENT 1. DCPA and Its Environmental Transformation Products. [A]

Code Name/ Synonym	Chemical Name	Chemical Structure	Study Type	MRID	Maximum %AR (day)		Final %AR (study length)
PARENT							
DCPA (Dacthal, Chlorthal-dimethyl; SDS-893)	**IUPAC:** Dimethyl 2,3,5,6-tetrachlorobenzene-1,4-dicarboxylate **CAS #:** 1861-32-1 **Formula:** $C_{10}H_6Cl_4O_4$ **MW:** 331.9 g/mol **SMILES:** COC(=O)c1c(c(c(c(c1Cl)Cl)C(=O)OC)Cl)Cl		835.4300 Aerobic aquatic metabolism	49307515	PRT		PRT
MAJOR (>10%) TRANSFORMATION PRODUCTS							
MTP (Dacthal monoacid, SDS-1449)	**IUPAC:** 2,3,5,6-Tetrachloro-4-methoxycarbonyl-benzoic acid **CAS #:** 887-54-7 **Formula:** $C_9H_4Cl_4O_4$ **MW:** 317.9 g/mol **SMILES:** COC(=O)c1c(c(c(c(c1Cl)Cl)C(=O)O)Cl)Cl		835.4300 Aerobic aquatic metabolism	49307515	River water:silty clay loam	**54.9%** (90 d)	**54.9%** (90 d)
TPA (Dacthal diacid, SDS-954)	**IUPAC:** 2,3,5,6-Tetrachloroterephthalic acid **CAS #:** 2136-79-0 **Formula:** $C_8H_2Cl_4O_4$ **MW:** 303.9 g/mol **SMILES:** c1(c(c(c(c(c1Cl)Cl)C(=O)O)Cl)Cl)C(=O)O		835.4300 Aerobic aquatic metabolism	49307515	River water:silty clay loam	**26.2%** (90 d)	**26.2%** (90 d)

Code Name/ Synonym	Chemical Name	Chemical Structure	Study Type	MRID	Maximum %AR (day)	Final %AR (study length)
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”. PRT means "parent".

Attachment 2: Statistics Spreadsheets and Graphs

Attachment 3: Calculations

Calculations were performed by the reviewer using PestDF, and the following equations.

Single First-Order (SFO) Model

$$C_t = C_0 e^{-kt} \qquad \text{(eq. 1)}$$

where,

C_t = concentration at time t (%)
C_0 = initial concentration (%)
e = Euler's number (-)
k = SFO rate constant of decline (d^{-1})
t = time (d)

The SFO equation is solved with R kinetics software by adjusting C_0 and k to minimize the objective function (S_{SFO}) shown in equation 9.

$$DT_{50} = \text{natural log } (2)/k \qquad \text{(eq. 2)}$$

$$DT_{90} = \ln(10)/k \qquad \text{(eq. 3)}$$

Indeterminate Order Rate Equation (IORE) Model

$$C_t = \left[C_0^{(1-N)} - (1-N)k_{IORE}t\right]^{\left(\frac{1}{1-N}\right)} \qquad \text{(eq. 4)}$$

where,

N = order of decline rate (-)
k_{IORE} = IORE rate constant of decline (d^{-1})

This equation is solved with R kinetics software by adjusting C0, kIORE, and N to minimize the objective function for IORE (SIORE) (See equation 9). Half-lives for the IORE model are calculated using equation 5, which represents a first-order half-life that passes through the DT_{90} of the IORE model. (Traditional DT_{50} and DT_{90} values for the IORE model can be calculated using equations 6 and 7.)

$$t_{IORE} = \frac{\log(2)}{\log(10)} \frac{C_0{}^{1-N}\left(1 - 0.1^{(1-N)}\right)}{(1-N)k_{IORE}} \qquad \text{(eq. 5)}$$

$$DT_{50} = \frac{(C_0/2)^{(1-N)} - C_0{}^{(1-N)}}{k(N-1)} \qquad \text{(eq. 6)}$$

$$DT_{90} = \frac{(C_0/10)^{(1-N)} - C_0^{(1-N)}}{k(N-1)}$$ (eq. 7)

Double First-Order in Parallel (DFOP) Model

$$C_t = C_0 g^{-k_1 t} + C_0(1-g)^{-k_2 t}$$ (eq. 8)

where,

g = the fraction of C_0 applied to compartment 1 (-)
k_1 = rate constant for compartment 1 (d^{-1})
k_2 = rate constant for compartment 2 (d^{-1})

If $C_0 \; x \; g$ is set equal to *a* and $C_0(1-g)$ is set equal to *c*, then the equation can be solved with R kinetics software for *a*, *c*, k_1, and k_2 by minimizing the objective function (S_{DFOP}) as described in equation 9.

DT_{50} and DT_{90} values can be calculated using equations 2 and 3, with k_1 or k_2 in place of k.

Objective Function: SFO, IORE, and DFOP are solved by minimizing the objective function (S_{SFO}, S_{IORE}, or S_{DFOP}).

$$S_{SFO}, S_{IORE}, \text{or } S_{DFOP} = \sum (C_{model}, t - C_{d,t})^2$$ (eq. 9)

where,

S_{SFO} , S_{IORE}, or S_{DFOP} = objective function of kinetics model fit ($\%^2$)
n = number of data points (-)
$C_{model,t}$ = modeled value at time corresponding to $C_{d,t}$ (%)
$C_{d,t}$ = experimental concentration at time t (%)

Critical Value to Determine Whether SFO is an Adequate Kinetics Model

If S_{SFO} is less than S_C, the SFO model is adequate to describe kinetics. If not, the faster of t_{IORE} or the DFOP DT_{50} for compartment 2 should be used.

$$S_c = S_{IORE}\left(1 + \frac{p}{n-p} F(\alpha, p, n-p)\right)$$ (eq. 10)

where,

S_c = the critical value that defines the confidence contours ($\%^2$)
p = number of parameters (3 in this case)
α = the confidence level (0.50 in this case)
F(α, p, n-p) = F distribution with α level of confidence and degrees of freedom p and n-p