Attachment 4

SUMMARIES OF SELECTED TRICLOSAN STUDIES FOR THE ITC

Volume 2

Prepared by William F. Salminen, Jr., Ph.D.
Ciba Specialty Chemicals Corporation
November 9, 2000

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BIODEGRADATION 1

Christensen, K.P. *Triclosan- Aerobic Biodegradation in Water.* Springborn Laboratories, Inc. Report Number 93-4-4731. April 12, 1994.

TRICLOSAN - AEROBIC BIODEGRADATION IN WATER

U.S FDA TECHNICAL ASSISTANCE DOCUMENT, SECTION 3.11

SUBMITTED TO:

Colgate-Palmolive Company
909 River Road
Piscataway, New Jersey 08855-1343

SLI Report # 93-4-4731

SLI Study # 1420.1292.6102.725

Study Director: Karen P. Christensen

Springborn Laboratories, Inc.
Environmental Sciences Division
790 Main Street
Wareham, Massachusetts 02571

12 April 1994

AMENDED FINAL REPORT

SUMMARY

NSOR: Colgate-Palmolive Company

TOCOL TITLE: "Protocol for Determining the Aerobic Biodegradation in Water Following FDA Technical Assistance Document 3-11", Springborn Laboratories Protocol #: 071692/FDA 3.11 and protocol amendments #1, 2 and 3 dated 16 December 1992, 19 January 1993 and 25 June 1993, respectively.

RT NUMBER: 93-4-4731

Y NUMBER: 1420.1292.6102.725

ARTICLE: Triclosan, Lot # EN 168236.96, a white powder, identified by the Study Sponsor to have a purity of 99.6%, was received from the Colgate-Palmolive Company on 20 November 1992.

^{14}C-Triclosan, Lot # 21738-037, which was determined to have a radiopurity of 94.8% and a specific activity of 15 μCi/mg was received from the Colgate-Palmolive Company on 22 October 1992.

RIMENTAL TEST S: 24 December 1992 - 19 February 1993

EDURES OWED: The carbon dioxide evolution method was coupled with liquid scintillation counting (LSC) to monitor ultimate biodegradation (mineralization), while high performance liquid chromatography with ultraviolet detection (HPLC-UV) was used to monitor the disappearance of Triclosan from the test system. The test was extended to 57 days because, although mineralization (to CO_2) of Triclosan was occurring, a plateau in $^{14}CO_2$ evolution had not been reached.

LTS: Results of LSC analysis are summarized in the following table:

	Mean Cumulative Percent Evolved[a]	
	$^{14}CO_2$	^{14}C-volatiles
Triclosan after 57 days	57.1%	10.2%
Glucose after 57 days	81.6%	0.1%

[a] Percent of material initially added, based on measured dpm

HPLC analysis indicated the complete disappearance of Triclosan by day 21 of testing.

NCLUSION: Based on the results, Triclosan degraded under the laboratory conditions maintained during this study. An average half-life of 5.2 days was determined for Triclosan. Substantial mineralization (81.6%) of the reference material (glucose) was recorded by test termination.

BIODEGRADATION 2

Christensen, K.P. *Triclosan- Determination of Anaerobic Aquatic Biodegradation.* Springborn Laboratories, Inc. Report Number 93-12-5076. April 14, 1994.

TRICLOSAN - DETERMINATION OF ANAEROBIC AQUATIC BIODEGRADATION

Submitted to:
Colgate-Palmolive Company
909 River Road
Piscataway, New Jersey 08855-1343

SLI Report #93-12-5076

Study # 1420.0493.6110.795

Study Director: Karen P. Christensen

Springborn Laboratories, Inc.
Environmental Sciences Division
790 Main Street
Wareham, Massachusetts 02571

14 April 1994

AMENDED FINAL REPORT

SUMMARY

Triclosan-
Determination of Anaerobic Biodegradation

SPONSOR:	Colgate-Palmolive Company
PROTOCOL TITLE:	"Determination of the Aquatic Biodegradation Potential of a Test Material Under Anaerobic Conditions," Springborn Laboratories Protocol #: 032493/Anaerobic Degradation, and protocol amendments #1, dated 26 May 1993, and #2, dated 20 June 1993.
REPORT NUMBER:	93-12-5076
SLI STUDY NUMBER:	1420.0493.6110.795
SPONSOR STUDY NUMBER:	C-P 93-028
TEST ARTICLE:	[^{14}C]Triclosan, Lot # 21738-037, identified by the Study Sponsor to have a specific activity of 15 μCi/mg, was received from the Colgate-Palmolive Company on 22 October 1992.
REFERENCE MATERIAL:	Ethanol (anhydrous), Lot # GG260, was received on 12 August 1991.
EXPERIMENTAL TEST DATES:	25 May to 20 October 1993.
RESULTS AND CONCLUSIONS:	The radioactivity distribution of extracts from test vessels receiving 200 μg/L Triclosan indicated that 90-92% of extractable residues remained as [^{14}C]Triclosan after 147 days of incubation with anaerobic inoculum, under anaerobic conditions. The percent extractable decreased steadily with time, but with no concomitant appearance of Triclosan metabolites it is unlikely that mineralization to CO_2 and methane occurred. Microbial analysis of the sludge during the study indicated that the sludge microbes were still viable.

Testing performed with a reference material, ethanol, in the same anaerobic test solution resulted in mean cumulative gas production of 69 to 310% of theoretical during the 147-day test, when corrected for blank gas production. The high gas production may have resulted from respiratory stimulation of the microbes by the ethanol, or by consumption of carbon contaminants in the glass bottles; nevertheless, the reference article performance indicates an active microbial community capable of degrading simple organic compounds.

BIODEGRADATION 3

Christensen, K.P. *Triclosan- Determination of Aerobic Biodegradation in Soils.* Springborn Laboratories, Inc. Report Number 93-5-4770. April 12, 1994.

TRICLOSAN - DETERMINATION OF AEROBIC BIODEGRADATION IN SOILS

U.S. FDA TECHNICAL ASSISTANCE DOCUMENT, SECTION 3.12

SUBMITTED TO:

Colgate-Palmolive Company
909 River Road
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SLI Report #: 93-5-4770
SLI Study #: 1420.1292.6103.730

Study Director: Karen P. Christensen

Springborn Laboratories, Inc.
790 Main Street
Wareham, Massachusetts 02571

12 April 1994

AMENDED FINAL REPORT

SUMMARY

Triclosan-
Determination of Aerobic Biodegradation in Soils

SPONSOR: Colgate-Palmolive Company

PROTOCOL TITLE: "Protocol for Conducting an Aerobic Biodegradation in Soil Study Following the FDA Technical Assistance Document, Section 3.12.", Springborn Laboratories Protocol #: 061990/FDA 3.12 and protocol amendments #1, dated 19 January 1993, #2, dated 9 February 1993, #3, dated 11 February 1993, #4, dated 28 June 1993 and #5, dated 21 July 1993.

REPORT NUMBER: 93-5-4770

STUDY NUMBER: 1420.1292.6103.730

TEST ARTICLE: ^{14}C-Triclosan, Lot # 21738-037, identified by the Study Sponsor to have a specific activity of 15 μCi/mg, was received from the Colgate-Palmolive Company on 22 October 1992.

REFERENCE MATERIAL: Glucose (dextrose), Lot # 916671A, was received on 2 March 1992.

^{14}C-Glucose, Lot # 2482-283, identified by the supplier to have a specific activity of 2.4 mCi/mmole and a radiochemical purity of 99.0%, was received on 27 October 1992.

EXPERIMENTAL TEST DATES: 4 February to 22 April 1993.

RESULTS AND CONCLUSIONS: The mean cumulative radiolabeled carbon dioxide evolution rates of soils fortified with ^{14}C-Triclosan were 20.1% for Arkansas silt loam, 11.9% for Kansas loam and 13.6% for Wisconsin sandy loam after 64 days of testing.

Chemical-specific analysis by HPLC-RAM indicated that primary biodegradation of Triclosan occurred, resulting in the loss of 77.3, 79.3 and 93.1% loss of Triclosan initially applied to the Arkansas,

Kansas and Wisconsin soils, respectively. Based on these results, the half-life of Triclosan was calculated as 35.2 days in Arkansas silt loam, 29.1 days in Kansas loam, and 17.4 days in Wisconsin sandy loam.

Testing performed with a reference material, glucose, in the same soil types resulted in mean cumulative radiolabeled carbon dioxide evolution rates of 50.5% for Arkansas silt loam, 42.6% for Kansas loam and 42.8% for Wisconsin sandy loam after 64 days of testing.

The results of this study indicated that Triclosan undergoes both primary biodegradation and complete mineralization under the conditions tested.

BIODEGRADATION 4

Marks, K.H., Crapo, K.C., Doi, J., and Marks, P.J. *$^{14}CO_2$ Production Test in Activated Sludge: D1063.02R..* Weston Study Number 90-069. June 11, 1992.

WESTON

R

THE PROCTER & GAMBLE COMPANY
CINCINNATI, OHIO

$^{14}CO_2$ PRODUCTION TEST
IN ACTIVATED SLUDGE

D1063.02R

WESTON Study Nº 90-069

Weston W.O. Nº 0211-24-01

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LIBRARIAN SECTION

1.0 EXECUTIVE SUMMARY

Roy F. Weston, Inc. (Weston) conducted a $^{14}CO_2$ production test in activated sludge on test substance D1063.02R. This test is designed to determine the rate and extent of ultimate biodegradation of the test substance by the activated sludge microflora.

The test apparatus consisted of thirteen (13) glass two-liter Erlenmeyer flasks containing 1100 mL of activated sludge collected from the Avondale Sewage Treatment Plant, Avondale, Pennsylvania. The sludge was screened through a 2 mm sieve. The total suspended solids (TSS) was determined and based on these results the sludge was dispensed to the test flasks at a solids level of 2500 mg/L. Sludge supernatant was used to dilute the sludge to the desired concentration.

Test substance D1063.02R was spiked to triplicate test flasks at the following concentrations: 20, 100, and 200 μg/L. ^{14}C Stearic acid was spiked to triplicate test flasks at a concentration of 20 μg/L. One (1) additional flask was spiked with D1063.02R at 100 μg/L and acidified with 10 mL concentrated hydrochloric acid (HCl) at test initiation.

The flasks were placed on a rotary platform shaker and aerated continuously with a CO_2-free air source. Following one (1) hour of mixing, 100 mL sludge samples were withdrawn from all flasks and acidified with 1 mL concentrated HCl. The samples were refrigerated until shipped to the Sponsor as requested in the protocol.

Samples collected on days specified on the protocol were assayed for ^{14}C activity by liquid scintillation counting (LSC). After 71 days, the contents of the flasks containing D1063.02R at 20 and 100 μg/L and ^{14}C stearic acid at 20 μg/L were acidified with concentrated HCl. One final set of samples was collected and analyzed. The remaining contents of these flasks

including the acidified control were sent to the Sponsor for analysis of residual radioactivity.

The flasks containing D1063.02R at a concentration of 200 μg/L were treated as follows:

a. 500 mL sludge was retained from each flask for additional spiking.
b. Two (2) of the three (3) flasks were spiked with an additional 1000 μg/L of D1063.02R. The third flask was used as a control.
c. Following termination, the contents of these flasks were shipped to the Sponsor.

The distribution of ^{14}C activity was calculated based on the results of the triplicate LS counts on the D1063.02R and ^{14}C stearic acid stock solutions analyzed on the day the test was initiated.

The cumulative percent (%) of the theoretical $^{14}CO_2$ ($T^{14}CO_2$) produced for the individual test flasks was as follows:

TSIN[1]	Flask N°	Concentration (μg/L)	Final $\%T^{14}CO_2$
D1063.02R	1	20	27.8
D1063.02R	2	20	29.2
D1063.02R	4	20	35.6
D1063.02R	5	100	45.6
D1063.02R	7	100	41.0
D1063.02R	8	100	47.4
D1063.02R[2]	9	200	50.8
D1063.02R[2]	10	200	55.7
D1063.02R[2]	11	200	50.4

[1] TSIN = Test Substance Identification Number

[2] Reflects $\%T^{14}CO_2$ data as of 31 November 1990 prior to the initiation of the respike study.

TSIN[1]	Flask Nº	Concentration (µg/L)	Final %T$^{14}CO_2$
^{14}C Stearic Acid	12	20	108.
^{14}C Stearic Acid	13	20	101.
^{14}C Stearic Acid	14	20	97.7
D1063.02R[2]	15	100	0.7

Respike Study: Protocol Amendment Nº 2

TSIN[1]	Flask Nº	Concentration (µg/L)	Final %T$^{14}CO_2$
Control[3]	9	1000	1.9[5]
D1063.02R[4]	10	1000	78.9
D1063.02R[4]	11	1000	80.9

Fractionation of the D1063.02R BAS systems indicated that most (> 82%) of the residual radioactivity was associated with the particulates. In biologically active systems, 31 to 69% of the total residual could be recovered from the solids with acetone. Rad-HPLC of the acetone extract indicated that this radioactivity consisted of 21 - 58% parent D1063.02R, 0-37% polar metabolites and 41-46% nonpolar metabolite. In the abiotic control, 71% of the total residual could be recovered from the solids with acetone, and Rad-HPLC analysis revealed that > 92% of this radioactivity consisted of parent D1063.02R. In general, the percentage of D1063.02R initially added, which was recovered from the solids, decreased

1 TSIN = Test Substance Identification Number

2 Test flask contents was acidified with 10 mL concentrated HCl at test initiation.

3 Flask was originally dosed with D1063.02R at 200 µg/L. During the respike study flask 9 was used as the control for flasks 10 and 11.

4 Reflects final %T$^{14}CO_2$ data following dosing with D1063.02R at 1000 µg/L.

5 Reflects the increase in CO_2 production from 31 July 1990 to 22 September 1990.

with increasing recovery of $^{14}CO_2$. In a like manner, the relative abundance of nonpolar metabolite decreased but that of polar metabolites increased with increasing recovery of $^{14}CO_2$.

2.0 INTRODUCTION

Roy F. Weston, Inc. (Weston) conducted a $^{14}CO_2$ production test in activated sludge on test substance D1063.02R. Many synthetic industrial chemicals are disposed to the sewer and are treated by municipal sewage treatment plants (STPs) or treated on-site at the manufacturing plant. Activated sludge is a common type of wastewater treatment process at STPs and at some manufacturing plants. Therefore, biodegradation of these test chemicals by the activated sludge microflora may be an effective removal mechanism.

3.0 OBJECTIVE

To determine the rate and extent of ultimate biodegradation of the test substance in activated sludge.

4.0 PROCEDURE

4.1 Test Set-Up. CO_2-free air was provided to the test flasks through a scrubbing apparatus prepared as follows:

- One empty 1-liter plastic bottle to prevent backflow into the air line.
- Five 1-liter plastic bottles containing 700 mL 10 N NaOH.
- One empty 1-liter plastic bottle to prevent overflow of alkali into the test containers.

The bottles were connected in series with Tygon tubing to a pressurized air source. Air was purged through the scrubbing train at a constant rate adequate to provide

BIODEGRADATION 5

Marks, K.H., Crapo, K.C., Doi, J., and Marks, P.J. *$^{14}CO_2$ Production Test in Sludge-Amended Soil: D1063.02R.*. Weston Study Number 90-070. May 26, 1994.

WESTON

$^{14}CO_2$ PRODUCTION TEST IN SLUDGE-AMENDED SOIL
D1063.02R

THE PROCTER & GAMBLE COMPANY
Cincinnati, Ohio

WESTON Study Nº 90-070

WESTON W.O. Nº 00211-024-005

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Fate and Effect Laboratory
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Lionville, Pennsylvania 19341-1345

1.0 EXECUTIVE SUMMARY

Roy F. Weston, Inc. (WESTON®) conducted a $^{14}CO_2$ production test in sludge-amended soil on test substance D1063.02R. This test was designed to determine the rate and extent of ultimate biodegradation of the test substance in sludge-amended agricultural soil.

The test apparatus consisted of 15 glass 125-mL Erlenmeyer flasks containing 15 g dry weight equivalent of soil at a water content of approximately 35%. Triplicate flasks were utilized for each of the following treatments:

TSIN[1]	Concentration (µg/kg)
D1063.02R	40
D1063.02R	600
D1063.02R ("Killed" Control)[2]	600
D1063.02R (Photolysis Control)[3]	600
^{14}C Stearic Acid	40

During the study, all flasks with the exception of the photolysis controls were aerated continuously with a CO_2-free air source. $^{14}CO_2$ evolved from the flasks was trapped in 1.5N KOH. Samples were periodically collected and assayed for ^{14}C activity by liquid scintillation counting (LSC). After 577 days, the contents of the flasks were acidified with 1N hydrochloric acid (HCl) and a final set of samples was collected from the KOH traps and analyzed.

[1]TSIN = Test Substance Identification Number

[2] Prior to spiking, the flasks containing soil only were autoclaved for ~2 hours; 3% Formalin was added. After spiking, the flasks were added to the test set up. No sampling was required during the test period.

[3]Prior to spiking, the flasks containing soil only were autoclaved for ~2 hours; 3% Formalin was added. After spiking, the flasks were placed outside for the entire test duration. No sampling was required during the test period.

The $^{14}CO_2$ evolved from the "killed controls" during the study was counted at test termination only. These flasks were not acidified or purged.

The cumulative percent (%) $T^{14}CO_2$ produced for the individual test flasks was as follows:

TSIN	Flask N°	Concentration (µg/kg)	Final %$T^{14}CO_2$
D1063.02R	15	40	53.2
D1063.02R	13	40	48.1
D1063.02R	6	40	51.8
D1063.02R	9	600	24.7
D1063.02R	4	600	26.3
D1063.02R	11	600	26.0
^{14}C Stearic Acid	1	40	87.4
^{14}C Stearic Acid	8	40	81.1
^{14}C Stearic Acid	2	40	86.1
"Killed" Control	7	600	0.6
"Killed" Control	3	600	0.8
"Killed" Control	5	600	0.6

Following the incubation period, the contents of the flasks were shipped to the Sponsor for analysis of residual radioactivity. The soil samples were lyophilized and extracted with acetone. The extracts were subsequently analyzed by Rad-TLC for parent D1063.02R and degradation products. In addition, subsamples of the extracted soil were combusted to quantitate the level of residual radioactivity associated with the soil solids, and other subsamples of the acetone extracted soil were extracted with base (0.5N NaOH) using the normal soil extraction procedure for humics and humic-like materials.

After the 577 day incubation, approximately half the ^{14}C parent test substance initially dosed was recovered as intact parent in the acetone extracts of the "killed" controls. In the case of the photolysis control, less than 14% of the dosed ^{14}C parent test substance was recovered intact, but an additional 6 to 25% of the initial radioactivity was recovered as a polar product, which presumably was photodegradation products(s). In the bioactive treatments,

0 to 1.3% of dosed ^{14}C parent test substance was recovered intact, and an additional 2.3 to 3.8% of the initial radioactivity was recovered as polar and nonpolar biodegradation products. In all treatments, a significant fraction (32 to 51%) of the added radioactivity remained bound to the soil. Although this bound test substance exhibited behavior consistent with that of the natural humics and fulvics, it was not possible to unequivocally demonstrate that it was not intact parent, which had not been extracted with acetone.

In summary, the sponsor's analytical results were consistent with the $^{14}CO_2$ data and confirm that D1063.02R was biodegraded in a sludge amended soil. Furthermore, they show that D1063.02R can undergo photodegradation during extended exposure to natural sunlight. During the 577 day incubation period, photodegradation and biodegradation resulted in 3-5 and 40-50 fold reductions, respectively, in the amount of D1063.02R, which could be extracted from soil with acetone. The total loss of D1063.02R was likely greater but not possible to determine accurately due to an inability to discriminate between bound parent and natural humics and fulvics.

2.0 INTRODUCTION

WESTON conducted a $^{14}CO_2$ production test in sludge-amended soil on test substance D1063.02R. Many synthetic chemicals may reside in soil as a result of their discharge from septic tank systems, amendments of sewage sludge to soil, or landfilling of industrial and consumer wastes. This test system was designed to determine the ultimate biodegradation of ^{14}C-labeled substrate in soil by monitoring $^{14}CO_2$ production.

3.0 OBJECTIVE

The objective of this study was to determine the rate and extent of ultimate biodegradation of the test substance in sludge-amended agricultural soil.

BIODEGRADATION 6

Bottcher, J. *Report on the Determination of the IC50 (Inhibitory Concentration) – OECD 209 – of FAT-80'023/Q.* Ciba Geigy, Ltd. Test Number G06905. March 12, 1990.

FINAL REPORT

CIBA-GEIGY Limited
D&C Product Ecotoxicology
FC 6.13

Test No.: G 069 05

R E P O R T

Report on the DETERMINATION of the IC_{50} (Inhibitory Concentration) - OECD 209 - of FAT-80'023/Q

Sponsor : CIBA-GEIGY Ltd., Basle, FC-Division 6.1

Test substance : FAT-80'023/Q

Test method : SOP: MBS 001 02

Additional identification

- Physical appearance: solid
- Expiry date : July 1992
- Batch No. : EN 91390.76 (Klybeck)
- Purity : According to the sponsor's file (analytical certificate)
- Characterization : by the sponsor

Date evaluated : 14.02.90

Study director : Mr. J. Böttcher

Personnel : Mr. E. Schmid

Reported : 12.03.90

Archives : CIBA-GEIGY Ltd., Basle, K-1363.U.219

1. SUMMARY

Study	: Determination of the IC_{50} (3 h) (Inhibitory concentration)
Substance	: FAT-80'023/Q
Guideline	: OECD Test Method 209, "Activated Sludge, Respiration Inhibition Test", April 4, 1984, correspondig to ETAD-Method No.103
Method	: SOP: MBS 001 02
Modification from the guideline	: Sludge from a Husman laboratory apparatus
Modification from the method	: none
Result	: IC_{50} (3 h) of FAT-80'023/Q: 20 mg/l

****

BIODEGRADATION 7

Marks, K.H., Crapo, K.C., Doi, J., and Marks, P.J. *Assessing the Removal of the Test Substance During Secondary Wastewater Treatment: D1063.01.* Weston Study Number 90-117. July 8, 1992.

WESTON

ASSESSING THE REMOVAL OF THE TEST SUBSTANCE DURING SECONDARY WASTEWATER TREATMENT

D1063.01

THE PROCTER & GAMBLE COMPANY
Cincinnati, Ohio

WESTON Study № 90-117

WESTON W.O. № 0211-24-07

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WESTON

ASSESSING THE REMOVAL OF THE TEST SUBSTANCE DURING SECONDARY WASTEWATER TREATMENT

D1063.01

1.0 EXECUTIVE SUMMARY

Roy F. Weston, Inc. (WESTON) conducted a continuous activated sludge (CAS) study on test substance D1063.01 to assess treatability during secondary wastewater treatment. The CAS testing was performed to give realistic estimates of removal during secondary wastewater treatment and to enable more accurate assessment of the environmental safety of the test substance. The study was divided into six (6) phases during which the concentration of test substance D1063.01 was incrementally increased from 40 μg/L to 2,000 μg/L. A seventh phase consisted of a testing period using unacclimated solids and a high level dosing experiment. Removals throughout all phases of the study were determined by the use of a ^{14}C radiolabelled form of the test substance (D1063.02R).

The test apparatus consisted of two (2) small mixing chambers which served to mix the incoming wastewater and test substance feed solutions before they entered the aeration basins. Two (2) aeration basins, each containing six (6) liters of activated sludge from the Avondale Sewage Treatment Plant, Avondale, Pennsylvania, were aerated by gas dispersion tubes using compressed air. The aeration basins discharged to individual 2-liter cylindrical clarifiers which were stirred by a shaft mixer at a speed of approximately (~) 2 rpm. The clarifiers had a recycle discharge in the bottom and a side tube for effluent discharge. The influent flow rate to the aeration basin was ~ 1,000 mL/hr (consisting of ~ 910 mL of wastewater and ~ 90 mL feed solution).

Primary stock solutions of labelled test substance (D1063.02R) were prepared at ~ 85 μCi/mL in deionized water at a pH of ~ 11.0. These solutions were assayed for ^{14}C and stored in the dark under refrigeration. Fresh solutions of the unlabelled test substance (D1063.01) were prepared weekly in deionized water at pH ~ 11.0.

Test feed solutions were prepared with a combination of labelled and unlabelled forms of the test substance to contain 5 μCi/L and a total of 11.1 times the nominal influent concentrations. The concentration of labelled test substance remained constant at 394 μg/L. Adjustments for the different concentrations were made using unlabelled test substance.

Nominal test substance concentrations in the influent to the test unit were incrementally increased at the following concentrations (μg/L): 40, 100, 200, 500, 1,000 and 2,000. The level of radioactivity in the influent remained constant at ~ 1,000 dpm/mL.

Following set-up, the CAS units were allowed to stabilize for eleven (11) days. During this stabilization period both units received wastewater and deionized water without test substance present.

Following the stabilization period, an acclimation period was conducted for each test substance concentration, lasting until removal of the test substance equilibrated. During acclimation, influent, aeration mixed liquor and effluent samples were collected and assayed for ^{14}C. Samples were not collected from the control unit for ^{14}C assay.

A five (5) day removal period was conducted for each test substance concentration following the respective acclimation period. During this time influent, effluent and aeration mixed liquor samples were collected and assayed for ^{14}C by WESTON. Influent, aeration mixed liquor and composite effluent samples were collected and shipped to the Sponsor for analysis and toxicity testing. The influent and effluent samples were analyzed by the Sponsor for parent test substance and metabolites using Rad HPLC. In addition, the level of radioactive parent and biomass associated with the solids was determined.

Throughout the study the performance of the units was monitored by measuring the following parameters: chemical oxygen demand (COD), ammonia (NH_3), nitrate nitrogen (NO_3), total suspended solids (TSS), biological oxygen demand (BOD_5), pH,

sludge volume index (SVI), sludge blanket height, and dissolved oxygen (DO). No adverse effects on the overall CAS system performance were observed during any phases of the study period.

During the individual removal periods, the mean overall removal, removal by biodegradation, and removal by sorption for test substance D1063.01 were as follows:

Phase	Concentration (µg/L)	Removal Range (%)	Mean Overall Removal (%)	Parent Removal[1] (%)	Removal by Biodegradation (%)[1]	Removal by Sorption[1] (%)
1	40	(75.5-89.8)	83.8 ± 5.9	> 99.5	74.7	9.1
2	100	(85.6-90.3)	88.0 ± 1.9	> 99.5	75.5	12.5
3	200	(91.0-94.3)	92.7 ± 1.5	> 99.5	85.7	7.0
4	500	(85.2-87.2)	86.1 ± 0.9	> 99.5	76.3	9.8
5	1,000	(91.5-94.7)	93.2 ± 1.2	> 99.5	87.8	5.4
6	2,000	(93.8-96.4)	95.4 ± 1.0	> 99.5	87.1	8.3

Following the incremental phase testing, a seventh phase was conducted to determine the biodegradation potential of the test substance in unacclimated solids and to determine its removability and biodegradation potential with periodic high level dosing.

The solids (both aeration chamber and clarifier solids) from test (Unit 5) and control (Unit 6) were switched. Dosing of test substance to unit 5 was initiated at 35 µg/L (labelled only). This procedure was performed to determine the biodegradation potential of the test substance in unacclimated solids. Solids were switched to utilize a unit that had already equilibrated to the test substance (Protocol Amendment Nº 4). The mean overall test substance removal in percent (%) during this period was 84.0 ± 2.2.

Unit 5 was subsequently dosed at 750 µg/L over a four hour period. Testing was then continued at 35 µg/L for two (2) days and the four hour dosing at 750 µg/L was

[1]Data is based on specific radiochemical analyses summarized in Tables 1 and 3 of the Analytical Report (Appendix F).

repeated. This procedure was performed to determine the removability and biodegradation potential of the test substance with periodic high level dosing (Protocol Amendment № 5).

The mean overall test substance removals in percent (%) for the two 750 μg/L dosing periods were 83.7 ± 1.8 and 84.6 ± 2.6, respectively. Specific analyses by the Sponsor indicated that removal of the parent ranged from 94 to 97% during this phase; furthermore, radioactivity recovered from the solids was 40 to 74% parent and 28 to 35% biomass constituents.

The performance of the units was monitored by measuring the following parameters: COD, TSS, BOD, and nitrogens. No adverse effects on the CAS system performance were observed during phase 7.

2.0 INTRODUCTION

WESTON conducted a continuous activated sludge (CAS) study on test substance D1063.01 to assess treatability during secondary wastewater treatment. The CAS testing was performed to give realistic estimates of removal during secondary wastewater treatment and to enable more accurate assessment of the environmental safety of the test substance. The study was divided into the following phases:

Stabilization

Phase 1:	Acclimation -	40 μg/L
	Removal -	40 μg/L
Phase 2:	Acclimation -	100 μg/L
	Removal -	100 μg/L
Phase 3:	Acclimation -	200 μg/L
	Removal -	200 μg/L
Phase 4:	Acclimation -	500 μg/L
	Removal -	500 μg/L

BIODEGRADATION 8

Marks, K.H., Crapo, K.C., and Doi, J. *Assessing the Fate of the Test Substance During Secondary Wastewater Treatment: Irgasan.* Weston Study Number 94-056. August 5, 1997.

WESTON

ASSESSING THE FATE OF THE
TEST SUBSTANCE DURING SECONDARY
WASTEWATER TREATMENT

IRGASAN

TRICLOSAN INDUSTRY ALLIANCE (TIA)
represented by Procter & Gamble and Ciba-Geigy

what is city & STATE for source wastewater

WESTON Study Nº 94-056

W.O. Nº 00892-027-001

Kay H. Marks 8/5/97
Kay H. Marks Date
Study Director

Kathleen C Crapo 8/5/97
Kathleen C. Crapo Date
Quality Assurance Coordinator

Jon Doi 8-5-97
Jon Doi, Ph.D. Date
Laboratory Manager

Testing Facility

ROY F. WESTON, INC.
Fate and Effect Laboratory
254 Welsh Pool Road
Lionville, Pennsylvania 19341-1345

WESTON

ASSESSING THE FATE OF THE TEST SUBSTANCE
DURING SECONDARY WASTEWATER TREATMENT

IRGASAN

1.0 EXECUTIVE SUMMARY

Roy F. Weston, Inc. (WESTON®) conducted a continuous activated sludge (CAS) study on a test substance identified as Irgasan. The study was performed to provide a realistic measurement of the fate of the test substance during secondary wastewater treatment and to enable more accurate assessment of the environmental safety of the test substance. The study was divided into three phases: (1) stabilization, (2) acclimation: unit 2 dosed with [^{14}C] Irgasan, and (3) sampling: unit 2 dosed with [^{13}C] Irgasan.

Two CAS units were required for this study. Units 1 and 2 were designated the control and test, respectively. The testing apparatus consisted of two small mixing chambers which served to mix the incoming wastewater and test substance feed solutions before they entered the aeration basins. Two aeration basins, each containing six liters of activated sludge from the Downingtown Regional Water Pollution Control Center (DRWPCC), were aerated by gas dispersion tubes using compressed air. The aeration basins discharged to individual two-liter cylindrical clarifiers which were stirred by a shaft mixing at approximately 2 rpm. The clarifiers had a recycle discharge in the bottom and a side tube for effluent discharge. The influent flow rate to each aeration basin was ~1,000 mL/hr (consisting of ~910 mL of wastewater and ~90 mL feed solution).

Following set-up, the CAS units were allowed to stabilize for 10 days. During this time, both units received wastewater and deionized water without test substance present. A one time collection of effluent, wastewater, and activated sludge was shipped to Prof. Christoffer Rappe at the University of Umeå in Umeå, Sweden.

Stabilization was followed by an acclimation phase lasting ~4 weeks during which time unit 2 was exposed to the C^{14} form of the test substance at a concentration of 10 μg/L with a radiochemical concentration of ~0.45 μCi/L. Each CAS unit received wastewater mixed with a feed solution of test substance (test unit) or deionized water (control unit). Percent removal was established by assaying the C^{14} level in the influent, effluent, and mixed liquor fractions. Based on the results of these analyses, the mean overall test substance removal in percent was 90.88.

Prior to initiation of the sampling phase, a one time collection of effluent, influent and aeration mixed liquor was shipped to Dr. T. Federle for the analysis of parent, metabolites, and incorporation into biomass. Based on the results of these analyses, the removal of parent Irgasan at the influent concentration of 10 μg/L was approximately 95%. A low level of Irgasan on the sludge solids combined with the presence of polar metabolites and extensive incorporation into biomass indicated that biodegradation rather than sorption was the major removal mechanism. Under the test conditions, the concentration of Irgasan in effluent was 0.5 μg/L and on solids was 3.71 mg/kg (Appendix D).

The dosing of radiolabelled Irgasan was discontinued on 10 February 1995 and for the remainder of the study unit 2 was exposed to the C^{13} form of the test compound at a concentration of 10 μg/L. Periodically during the next three weeks, the mixed liquor in the test unit aeration basin was monitored for the disappearance of C^{14}.

On 7 March 1995, the sampling period was initiated. On three separate days during the following nine days, effluent and activated sludge samples were collected and shipped to both Prof. Rappe in Sweden and Mr. M. Jacob of Ciba-Geigy AG in Basel, Switzerland. In addition and independent of the CAS units, an influent solution was prepared and shipped to both Prof. Rappe and Mr. Jacob.

Throughout the study, the performance of the CAS units was monitored by measuring the following parameters: chemical oxygen demand (COD), total suspended solids (TSS), pH,

sludge volume index (SVI), sludge blanket height, dissolved oxygen (DO), and flow rates. No adverse effects on the overall CAS system performance were observed during any phase of the study.

2.0 INTRODUCTION

The CAS test is used to provide a realistic measurement of the fate of a test substance during secondary wastewater treatment and to enable more accurate assessment of the environmental safety of a test substance. The test system was activated sludge collected from an aeration basin located at the DRWPCC. The study was divided into the following phases: stabilization, acclimation, and sampling.

The stabilization phase allowed the mixed liquor to adjust to laboratory conditions. The purpose of acclimation was to pre-expose the microbial population to the radiolabelled form of the test substance and through LSC analysis to determine the appropriate time to initiate the sampling phase. Settling characteristics, suspended solids removal, and mixed liquor solids were evaluated for signs of potential toxicity due to test substance addition during this phase. The following parameters were monitored throughout the study: TSS, pH, COD, SVI, blanket height, DO, and flow rates.

3.0 OBJECTIVE

The objective of this study was to measure the fate of the test substance in a bench-scale CAS unit receiving domestic wastewater.

BIODEGRADATION 9

Marks, K.H., Crapo, K.C., and Doi, J. *Assessing the Removal of the Test Substance During Secondary Wastewater Treatment: Irgasan DP300.* Weston Study Number 97-018. March 16, 1998.

received
13 April 98

ASSESSING THE REMOVAL OF THE
TEST SUBSTANCE DURING SECONDARY
WASTEWATER TREATMENT

IRGASAN DP 300

FINAL REPORT

CIBA SPECIALTY CHEMICALS
410 Swing Road
Greensboro, NC

WESTON Study Nº 97-018

W.O. Nº 00892-028-001

Kay H. Marks 3/16/98
Kay H. Marks Date
Study Director

Kathleen C Crapo 3/15/98
Kathleen C. Crapo Date
Quality Assurance Coordinator

Jon Doi 3-16-98
Jon Doi, Ph.D. Date
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Testing Facility

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Fate and Effect Laboratory
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ASSESSING THE REMOVAL OF THE TEST SUBSTANCE
DURING SECONDARY WASTEWATER TREATMENT

Irgasan DP 300

1.0 EXECUTIVE SUMMARY

Roy F. Weston, Inc. (WESTON®) conducted a continuous activated sludge (CAS) study on a test substance identified as Irgasan DP 300. The CAS testing was performed to give realistic estimates of removal during secondary wastewater treatment and to enable more accurate assessment of the environmental safety of the test substance. The study was divided into three phases: (1) set-up/stabilization, (2) equilibration, and (3) testing.

Five CAS units were required for this study. Unit 1 was designated the control. Units 2 through 5 were dosed at total nominal influent concentrations of 7.5, 11.0, 20.0, and 50.0 μg/L, respectively, assuming a background concentration of 6 μg/L. Each testing unit had a small mixing chamber which served to mix the incoming wastewater and test substance feed solutions before they entered the aeration basins. Six liters of activated sludge from the Downingtown Regional Water Pollution Control Center (DRWPCC) was placed into the aeration basins and aerated by gas dispersion tubes using compressed air. The aeration basins discharged to individual 2-liter cylindrical clarifiers which were stirred by a shaft mixing at approximately 2 rpm. The clarifiers had a recycle discharge in the bottom and a side tube for effluent discharge. The influent flow rate to each aeration basin was ~1,000 mL/hr (consisting of ~910 mL of wastewater and ~90 mL feed solution).

Following set-up, all CAS units received DRWPCC primary influent (wastewater) only for ~24 hours. This was followed by a two week stabilization period during which the test units received wastewater mixed with Irgasan DP 300 (unlabeled only) at the nominal influent concentrations of 1.5, 5.0, 14.0, and 44.0 μg/L, respectively. Throughout the study, the control unit received wastewater and deionized water at the same flow rate as the

test substance feed solutions. The control unit served to monitor the overall performance of the CAS process.

Stabilization was followed by an equilibration period during which the test units received wastewater mixed with the radiolabeled form of Irgasan DP 300 at concentrations of 1.5, 2.75, 5.0, and 12.5 μg/L, respectively. These concentrations were equivalent to approximate radiochemical concentrations of 0.234, 0.429, 0.780, and 1.95 μCi/L, respectively. Addition of the unlabeled form of Irgasan DP 300 continued at the adjusted concentrations of 0, 2.25, 9.00, and 31.5 μg/L, respectively. After an equilibration period of approximately one month, a testing phase was conducted for ~one week.

Percent removal was established by assaying the ^{14}C level in the test feed, effluent, influent, and mixed liquor fractions. Disappearance of ^{14}C parent test substance and the formation of any metabolites was determined by thin layer chromatography (RAD-TLC) analysis. The sludge solids were combusted to determine the amount of radioactivity remaining with the solids.

Throughout the study, the performance of the units was monitored by measuring the following parameters: chemical oxygen demand (COD), total suspended solids (TSS), nitrate nitrogen (NO_3), ammonia (NH_3), pH, sludge volume index (SVI), sludge blanket height, dissolved oxygen (DO), and flow rates. No adverse effects on the overall CAS system performance were observed during any phase of the study.

The following table shows the percent removal of both total and parent radioactivity:

Irgasan DP 300 @	7.5 μg/L	11.0 μg/L	20.0 μg/L	50.0 μg/L
% Removal: Total Radioactivity				
Mixing chamber	-1.59 ± 6.11	-11.03 ± 4.89	-20.44 ± 6.03	-9.96 ± 4.33
Total CAS System	86.86 ± 1.23	87.55 ± 0.62	82.48 ± 2.64	88.81 ± 1.35
% Removal: Parent				
Mixing chamber	-18.20 ± 17.63	-19.89 ± 22.30	-18.36 ± 7.69	-28.14 ± 18.50
Total CAS System	98.21 ± 0.42	99.33 ± 0.49	99.05 ± 0.26	99.09 ± 0.28

The following tables show fractions in various compartments as a percent of initial dosed radioactivity:

Irgasan DP 300 @ 7.5 μg/L	Mean ± SD (%)	Mean ± SD (μg/L)
Mineralization ($^{14}CO_2$)[1]	73.86 ± 2.27	not applicable
Remaining with the solids	17.01 ± 1.89	1.28 ± 0.14
Sorbed onto solids as:		
Parent	1.78 ± 0.39	0.13 ± 0.03
Metabolite:		
-0.022	0.20 ± 0.06	0.02 ± 0.00
0.028	1.07 ± 0.25	0.08 ± 0.02
0.751	0.98 ± 0.14	0.07 ± 0.01
Total Sorbed	4.04 ± 0.64	0.31 ± 0.05
Present in effluent as:		
Parent	1.45 ± 0.37	0.11 ± 0.03
Metabolite:		
-0.029	0.54 ± 0.28	0.04 ± 0.02
0.019	1.68 ± 0.35	0.13 ± 0.03
0.161	0.91 ± 0.23	0.07 ± 0.02
0.714	0.50 ± 0.07	0.04 ± 0.01
Total Effluent	5.09 ± 0.31	0.39 ± 0.03
% of Dosed Radioactivity undergoing		
Primary biodegradation[2]	96.77 ± 0.12	not applicable
Ultimate biodegradation[3]	90.87 ± 0.89	not applicable

[1] 100-(% remaining with solids + % in effluent + % sorbed)
[2] 100-(% parent sorbed + % parent in effluent)
[3] Mineralization (%) + incorporated into biomass (%)

Irgasan DP 300 @ 11.0 µg/L	Mean ± SD (%)	Mean ± SD (µg/L)
Mineralization ($^{14}CO_2$)	75.61 ± 2.41	not applicable
Remaining with the solids	15.81 ± 2.17	1.74 ± 0.24
Sorbed onto solids as:		
Parent	1.92 ± 0.11	0.21 ± 0.01
Metabolite:		
-0.030	0.14 ± 0.03	0.02 ± 0.00
0.015	1.37 ± 0.17	0.15 ± 0.02
0.653	0.58 ± 0.05	0.06 ± 0.01
Total Sorbed	4.01 ± 0.32	0.45 ± 0.03
Present in effluent as:		
Parent	0.64 ± 0.42	0.07 ± 0.05
Metabolite:		
-0.025	1.00 ± 0.18	0.11 ± 0.02
0.031	1.90 ± 0.33	0.21 ± 0.04
0.143	0.70 ± 0.16	0.08 ± 0.02
0.768	0.27 ± 0.07	0.03 ± 0.01
Total Effluent	4.58 ± 0.39	0.51 ± 0.06
% of dosed radioactivity undergoing		
Primary biodegradation	97.40 ± 0.39	not applicable
Ultimate biodegradation	91.41 ± 0.40	not applicable

Irgasan DP 300 @ 20.0 µg/L	Mean ± SD (%)	Mean ± SD (µg/L)
Mineralization ($^{14}CO_2$)	76.61 ± 1.75	not applicable
Remaining with the solids	14.20 ± 0.76	2.84 ± 0.15
Sorbed onto solids as:		
Parent	1.94 ± 0.37	0.39 ± 0.07
Metabolite:		
-0.031	0.09 ± 0.03	0.02 ± 0.01
0.016	0.82 ± 0.12	0.16 ± 0.02
0.694	0.48 ± 0.06	0.10 ± 0.01
Total Sorbed	3.34 ± 0.56	0.68 ± 0.11
Present in effluent as:		
Parent	0.90 ± 0.23	0.18 ± 0.05
Metabolite:		
-0.018	1.01 ± 0.33	0.20 ± 0.07
0.033	2.46 ± 0.50	0.49 ± 0.10
0.149	1.22 ± 0.48	0.24 ± 0.10
0.741	0.33 ± 0.07	0.07 ± 0.01
Total Effluent	5.86 ± 1.17	1.18 ± 0.23
% of dosed radioactivity undergoing		
Primary biodegradation	97.16 ± 0.61	not applicable
Ultimate biodegradation	90.80 ± 1.62	not applicable

Irgasan DP 300 @ 50.0 μg/L	Mean ± SD (%)	Mean ± SD (μg/L)
Mineralization ($^{14}CO_2$)	76.72 ± 2.45	not applicable
Remaining with the solids	16.08 ± 2.17	8.04 ± 1.09
Sorbed onto solids as:		
Parent	1.38 ± 0.14	0.69 ± 0.07
Metabolite:		
-0.031	0.14 ± 0.04	0.07 ± 0.02
0.012	1.45 ± 0.31	0.73 ± 0.15
0.694	0.22 ± 0.04	0.11 ± 0.02
Total Sorbed	3.20 ± 0.51	1.61 ± 0.26
Present in effluent as:		
Parent	0.72 ± 0.21	0.36 ± 0.11
Metabolite:		
-0.023	0.73 ± 0.14	0.37 ± 0.07
0.032	1.84 ± 0.29	0.92 ± 0.15
0.147	0.64 ± 0.21	0.32 ± 0.10
0.682	0.05 ± 0.06	0.03 ± 0.03
Total Effluent	4.00 ± 0.60	2.00 ± 0.29
% of dosed radioactivity undergoing		
Primary biodegradation	97.90 ± 0.28	not applicable
Ultimate biodegradation	92.80 ± 1.11	not applicable

For the 7.5 μg/L treatment:

RAD-TLC analysis of the mixed liquor solids extracts revealed that ~1.8% of the dosed radioactivity was present on the solids as parent (Rf 0.416). The other activity in the extract was divided among two unidentified polar peaks at Rfs -0.022 and 0.028 and a non-polar peak at Rf 0.751. The remaining radioactivity was associated with the solids. Combustion of extracted abiotic sludge solids spiked with parent indicated ~4% of the spike behaved as biomass constituents.

RAD-TLC analysis of the effluent extracts revealed ~1.5% of the dosed radioactivity was present as parent in the effluent. This equates to a 98.21% removal of parent equivalent to an effluent concentration of 0.11 μg/L. The remaining activity in the effluent was divided among three unidentified polar peaks at Rfs -0.029, 0.019, and 0.161 and a non-polar peak at Rf 0.714.

For the 11.0 μg/L treatment:

RAD-TLC analysis of the mixed liquor solids extracts revealed that ~1.9% of the dosed radioactivity was present on the solids as parent (Rf 0.370). The other activity in the extract was divided among two unidentified polar peaks Rfs -0.030 and 0.015 and a non-polar peak at Rf 0.653. The remaining radioactivity was associated with the sludge solids. Combustion of extracted abiotic solids spiked with parent indicated ~2.5% of the spike behaved as biomass constituents.

RAD-TLC analysis of the effluent extracts revealed 0.64% of the dosed radioactivity was present as parent in the effluent. This equates to an 99.33% removal of parent equivalent to an effluent concentration of 0.07 μg/L. The remaining activity in the effluent was found in three unidentified polar peaks at Rfs -0.025, 0.031, and 0.143 and in a non-polar peak at Rf 0.768.

For the 20 μg/L treatment:

RAD-TLC analysis of the mixed liquor solids extracts revealed that ~1.9% of the dosed radioactivity was present on the solids as parent (Rf 0.378). The other activity in the extract was divided among two unidentified polar peaks at Rfs -0.031 and 0.016 and a non-polar peak at Rf 0.694. The remaining radioactivity was associated with the solids. Combustion of extracted abiotic sludge solids spiked with parent indicated ~4% of the spike behaved as biomass constituents.

RAD-TLC analysis of the effluent extracts revealed 0.90% of the dosed radioactivity was present as parent in the effluent. This equates to a 99.05% removal of parent equivalent to an effluent concentration of 0.18 μg/L. The remaining activity in the effluent was divided among three unidentified polar peaks at Rfs -0.018, 0.033, and 0.149 and a non-polar peak at Rf 0.741.

For the 50.0 μg/L treatment:

RAD-TLC analysis of the mixed liquor solids extracts revealed that ~1.4% of the dosed radioactivity was present on the solids as parent (Rf 0.382). The other activity in the extract was divided among two unidentified polar peaks Rfs -0.031 and 0.012 and a non-polar peak at Rf 0.694. The remaining radioactivity was associated with the sludge solids. Combustion of extracted abiotic solids spiked with parent indicated ~4% of the spike behaved as biomass constituents.

RAD-TLC analysis of the effluent extracts revealed 0.72% of the dosed radioactivity was present as parent in the effluent. This equates to an 99.09% removal of parent equivalent to an effluent concentration of 0.36 μg/L. The remaining activity in the effluent was found in three unidentified polar peaks at Rfs -0.023, 0.032, and 0.147 and in a non-polar peak at Rf 0.682.

2.0 INTRODUCTION

The CAS test is used to provide realistic estimates of removal during secondary wastewater treatment and to enable more accurate assessment of the environmental safety of the test substance. In this study, a single test substance was tested at four influent concentrations. The test system was activated sludge collected from an aeration basin located at the DRWPCC.

Known concentrations of ^{14}C-labeled test substance (supplemented with the unlabeled form of the material as needed) were mixed with wastewater in reaction vessels (mixing chambers) which overflowed at a rate of 1,000 mL per hour into aeration basins containing six liters of activated sludge. The hydraulic residence time (HRT) in the mixing chamber was 0.5 hour. The sludge overflowed to clarifiers stirred at approximately two rpm. Sludge solids settled and were periodically recycled to the aeration basin. Effluent in the clarifier discharged to the sewer unless collected for analysis.

ADSORPTION 1

Heim, L.G. *Adsorption of ^{14}C-Triclosan to Suspended Solids.* ABC Laboratories Final Report Number 43356. April 22, 1997.

received
3 Nov 97

SUBMITTED TO

Ciba-Geigy Corporation
410 Swing Road
Greensboro, North Carolina 27409

STUDY TITLE

Adsorption of ^{14}C-Triclosan to Suspended Solids

GUIDELINE

U.S. Food and Drug Administration
Technical Assistance Document 3.08, Sorption/Desorption

AUTHOR

Lucas G. Heim
Study Director

STUDY COMPLETED ON

April 22, 1997

PERFORMING LABORATORY

ABC Laboratories, Inc.
Environmental Fate and Assessment
7200 E. ABC Lane
Columbia, Missouri 65202

ABC LABORATORIES' PROJECT ID

Final Report #43356

ABSTRACT

An adsorption study was conducted at 20 ± 2 °C with the test chemical, ^{14}C-triclosan, using deactivated sludge solids as the sorbent matrix at low total organic carbon (TOC) levels. The test was patterned after the methods outlined in the FDA Technical Assistance Document 3.08, Sorption/Desorption. The aqueous phase consisted of ABC Laboratories' well water buffered in the range of pH 6.5 - 7.0 using approximate 1 mM phosphate buffer. All phases of the study were conducted in triplicate at a solids concentration of approximately 50 mg/L. The nominal concentration of test chemical used for the preliminary tests was 250 μg/L. The nominal concentrations used to develop the Freundlich isotherm were 250, 100, 40, and 15 μg/L.

During the preliminary test, the minimum equilibration time was determined by sampling after 1, 2, 4, 8, 16, and 24 hours of equilibration. LSC analysis of the supernatants showed that equilibration was achieved by 8-hours. HPLC analysis of 24-hour adsorption supernatants showed that the test chemical was stable through the entire 24 hour equilibration. Adsorption of the test substance to the solid matrix was determined to be 52.2 percent at 8-hours. The K_d and K_{oc} values were estimated to be 19,775 and 49,438, respectively. Adsorption to the teflon centrifuge tubes was measured to be < 5 percent at 8-hours. The ^{14}C-mass balance for the test and control samples was 98.3 and 95.5 percent, respectively.

Based on the preliminary test results, and 8-hour equilibration was used to determine the Freundlich isotherm. The adsorption isotherm was observed to be linear (correlation coefficients ≥ 0.95). Since a linear fit was observed, the Freundlich model was applied. The definitive test adsorption results are summarized as follows:

Solids Concentration	49.6 mg/L
Percent Organic Carbon	45.5%
Initial Solids TOC	22.6 mg C/L
^{14}C-Mass Balance	97.0%
Percent Adsorbed	53.7%
Kd	21,592
Koc	47,454
n	1.02
correlation	0.9978

HPLC analysis of aqueous adsorption phases from the highest concentration verified stability of the test chemical under the conditions of the isotherm test. The identity of ^{14}C-triclosan in representative control and test sample supernatants was confirmed by co-chromatography with ^{14}C-triclosan and ^{12}C-triclosan standards.

ABC LABS #43356-10

The pH of the solids suspension was measured to be 6.55 at initiation. After 8-hours of equilibration, the pH of the test, blank, and control samples ranged from 6.79 to 6.98. The initial TOC of the solids suspension was measured to be 0.910 mg C/L. After 8-hours of equilibration, the test sample TOC ranged from 3.03 to 4.40 mg C/L.

Based on these results, the adsorption Koc value of 47,454 for ^{14}C-triclosan suggests that a portion of the test chemical will be sorbed to waste water treatment plant solids in the effluent.

ABC LABS #43356-11

ADSORPTION 2

Bottcher, J. and Matzler, P. *Report on the Leaching Behaviour of FAT-80'023/H (^{14}C) in Soil.* Ciba-Geigy Ltd. Textile Dyes and Chemicals Division Product Ecology Test Number G04511. April 18, 1994.

CIBA-GEIGY Ltd.
Textile Dyes and Chemicals Division
Product Ecology
CH-4002 Basle

ciba

Test No.: G 045 11

REPORT on the leaching behaviour of FAT - 80'023/H (^{14}C) in soil

Translation of the original report in german language (April 18, 1994)

Sponsor	:	CIBA-GEIGY Ltd. Textile Dyes Division represented by J. Inauen CH-4002 Basle
Test substance	:	FAT - 80'023/H (^{14}C)
Internal method	:	SOP: MBD 001 01
Additional identification		
- Apperance	:	solid
- Purity	:	According to the sponsor's files (Analytical certificate)
- Characterisation	:	by the sponsor
Experimental starting date	:	May 31, 1988
Experimental termination date	:	June 02, 1988
Study director	:	Mr. J. Böttcher
Technical Personel	:	Mr. P. Mätzler
Study completion date	:	November 27, 1989
Archives	:	CIBA-GEIGY Ltd. CH-4002 Basle, K-411.10.08

1. Summary

Study	:	Determination of the leaching behaviour in soil
Test substance	:	FAT - 80'023/H (^{14}C)
Guideline	:	"Versickerungsverhalten von Pflanzenschutzmittel" Dezember 1986, (4-2) Biologische Bundesanstalt für Land- und Forstwirtschaft, BRD
Method	:	SOP : MBD 001 01
Deviation from the Guideline	:	The test substance was adsorbed onto the sludge and this mixture was given into the test system.
Deviation from the method	:	Recovery: < 90 %

Results :

Eluate :

The total activity of labelled test substance in the fractions was smaller than the background, so the test substance was not leached out of the soil column within 48 hours.

All values are summarized in table 1.

Soil sample :

Between 88 - 95 % of the quantitity of FAT - 80'023/H (^{14}C) were present in the highest 5 cm of the soil column.
1 - 2 % of FAT - 80'023/H (^{14}C) were found in a depth of 5 - 10 cm of the soil column.
No test substance was detected below 15 cm.

All values are summarized in table 2.

ECOLOGICAL 1

Wuthrich, V. *48-Hour Acute Toxicity of FAT 80'023/Q to Daphnia Magna (OECD-Immobilization Test).* RCC Project Number 262923. November 9, 1990.

3.29

R C C

U M W E L T C H E M I E

ENGAGE
RECEIVED
DEC 6 1990
SEAD

Study Title:

48-HOUR ACUTE TOXICITY OF FAT 80'023/Q TO DAPHNIA MAGNA (OECD-IMMOBILIZATION TEST)

Data Requirement:

OECD Guideline No. 202, Part I
Directive 84/449 EEC, Part C.2

Author:

Mrs. V. Wüthrich

Study Completion Date:

November 9, 1990

Performing Laboratory:

R C C UMWELTCHEMIE AG
P.O. Box
CH - 4452 Itingen/BL
Switzerland

Study Project No. :

RCC PROJECT 262923

Page 1 of 36

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SUMMARY

FAT 80'023/Q was tested for its acute toxicity to Daphnia magna.
The tests were performed according to OECD Guideline 202, Part I and in compliance with EEC Directive 84/449.

The EC 0 (NOEC), EC 50 and EC 100 values after 48 hours of exposure were found to be 0.156 mg/l, 0.427 mg/l and 2.5 mg/l, respectively.

In conclusion, FAT 80'023/Q showed high toxicity to daphnids when exposed for 48 hours.

The immobilization observed with the control animals was 0 % after 48 hours. The tests were validated by testing potassium dichromate. The EC 50 values determined for potassium dichromate after 24 and 48 hours were 1.61 and 1.25 mg/l, respectively.

The analytical results can be summarized as follows:

Nominal concentration (mg/l)	Time (hours)	% of nominal
0.039	0	---
	48	31.6
0.625	0	77.3
	48	36.4
10	0	24.6
	48	67.0

ECOLOGICAL 2

Forbis, A.D. and Muckerman, J.G. *Acute Toxicity of D1063.01 (Triclosan; Irgasan® DP300) to Daphnia Magna.* ABC Laboratories Project Number 38656. July 25, 1990.

VOLUME 3 OF 3 OF SUBMISSION

STUDY TITLE

Acute Toxicity of D1063.01 (Triclosan; Irgasan® DP 300)
to Daphnia Magna

EPA GUIDELINE NO. 72-2

AUTHORS

Alan D. Forbis, Supervisor Aquatic Toxicology
James G. Muckerman, Biological Technician III

STUDY COMPLETED ON: July 25,1990

CONDUCTED BY

Analytical Bio-Chemistry Laboratories, Inc.
7200 East ABC Lane
P.O. Box 1097
Columbia, Missouri 65205

LABORATORY PROJECT IDENTIFICATION NO. 38656

PAGE 1 OF 47

SPONSOR

The Procter and Gamble Company
Spring Grove and June Street
Cincinnati, OH 45217

SUMMARY

The acute toxicity of D1063.01 to *Daphnia magna* was assessed using the methods outlined in Procter and Gamble's protocol entitled "Static Acute Freshwater Invertebrate Toxicity Study of D1063.01" under ABC study number 38656.

The study was conducted from May 31, 1990 to June 2, 1990 at the following nominal concentrations based on 99.7% active ingredient: 0.10, 0.18, 0.32, 0.56 and 1.0 mg/L. Twenty daphnids (<24-hour old) were exposed to each test concentration, the control and solvent control with four replications containing five daphnids each. The results of the 48-hour static *Daphnia magna* toxicity study are summarized below.

Compound	48-Hour LC_{50} (95% C.I.)	48 Hour Dose Response Slope
D1063.01	0.39 mg/L (0.33 - 0.46 mg/L)	4.7

Abnormal effects of mortality, quiescence, and/or daphnids tending to the bottom of the test vessels were observed in the 0.18, 0.32, 0.56, and 1.0 mg/L test concentrations. The control, solvent control and 0.10 mg/l (Level 1) were normal throughout the study. All solutions were observed to be clear for the duration of the study.

Water quality parameters of temperature, dissolved oxygen and pH were measured at 0, 24 and 48 hours of the test and were within acceptable limits. The test was conducted in ABC hard blended water of 160 mg/L hardness and 174 mg/L alkalinity (as $CaCO_3$) with conductivity of 320 µMhos/cm. The test temperature ranged from at 20 to 21°C. The dissolved oxygen and pH levels ranged from 8.1 to 8.5 mg/L and 7.9 to 8.5, respectively, during the 48-hour study.

ECOLOGICAL 3

Jop, K.M. *Toxicity Evaluation of D1063.02R and D1063.01 Using Ceriodaphnia dubia Under Static Conditions.* Springborne Laboratories, Inc. Report Number 91-10-3962. December 20, 1991.

TOXICITY EVALUATION OF D1063.02R and D1063.01 USING *Ceriodaphnia dubia* UNDER STATIC CONDITIONS

Submitted To:
The Procter and Gamble Company
Ivorydale Technical Center
5299 Spring Grove Avenue
Cincinnati, Ohio 45217

SLI report #91-10-3962

SLI Study #1011-0691-6278-132

STUDY DIRECTOR: Krzysztof M. Jop

Springborn Laboratories, Inc.
Environmental Sciences Division
790 Main Street
Wareham, Massachusetts 02571

20 December 1991

FINAL REPORT

COPY SENT

DATE:

EXECUTIVE SUMMARY

The purpose of this study was to determine the acute toxicity of D1063.02R and D1063.01 to *Ceriodaphnia dubia* at four pH levels. The acute toxicity tests were conducted at the following targeted pH: 7.0 ±0.1, 7.5 ±0.1, 8.0 ±0.1 and 8.5 ±0.1.

Biological response of *C. dubia* to D.1063.02R and D1063.01 was pH dependent. Within the tested pH range (7.0 - 8.5) the acute toxicity was three times lower in slightly basic solution (pH 8.5) then in the neutral solution (pH 7.0). The 48-hour LC50 at pH 7.0, 7.5, 8.0 and 8.5 was 0.13 mg/L, 0.18 mg/L, 0.24 mg/L, and 0.41 mg/L, respectively.

1.0 INTRODUCTION

The objective of this study was to measure the acute toxicity of D1063.02R and D1063.01 to *Ceriodaphnia dubia* under static test conditions. The acute toxicity of D1063.02R and D1063.01 was defined using 48-hour static acute toxicity test with *Ceriodaphnia dubia*. Each toxicity test was conducted at constant pH level of 7.0, 7.5, 8.0 and 8.5. The definitive toxicity tests were conducted between August 5 and September 12, 1991 at the Environmental Sciences Division of Springborn Laboratories, Inc. (SLI), in Wareham, Massachusetts. All original raw data and the final report produced during this study are stored at SLI.

ECOLOGICAL 4

Bottcher, J. *Report on the Acute Toxicity (96h)- OECD 203 – of FAT-80'023/Q to Zebrafish.* Ciba-Geigy LTd. D&C Product Ecotoxicology Test Number G06904. June 15, 1990.

FINAL REPORT

CIBA-GEIGY Limited
D&C Product Ecotoxicology
FC 6.13

Test No.: G 069 04

R E P O R T

Report on the acute toxicity (96h) - OECD 203 - of FAT-80'023/Q to Zebrafish

---

Sponsor : CIBA-GEIGY Ltd., Basle, FC-Division 6.1

Test substance : FAT-80'023/Q

Test method : SOP: MFI 003 02

Additional identification

- Physical appearance: solid
- Expiry date : July 1992
- Batch No. : EN 91390.76 (Klybeck)
- Purity : According to the sponsor's files (analytical certificate)
- Characterization : by the sponsor

Test begin : 23.04.90

Test end : 27.04.90

Study director : Mr. J. Böttcher

Personnel : Mr. J. Popovic / Mr. J. Böttcher

Analysis : 12.06.90 / Analytical Services K, FO 3.31

Reported : 15.06.90

Archives : CIBA-GEIGY Ltd., Basle, K-1363.U.219

1. SUMMARY

Study : Determination of the LC_{50} = concentration where 50 % of fish died.

Substance : FAT-80'023/Q

Organism : Zebrafish (Brachydanio rerio)

Duration : 96 h

Temperature : 21,4 - 24,1°C

Guideline : EEC Directive, Annex V, C.1, corresponding to OECD Guideline for testing chemicals No. 203, Paris 1984

Method : SOP: MFI 003 02

Modification from the guideline : - Holding : in dechlorinated tap water
- Test : in dechlorinated tap water
- Temperature: higher temperatur variation

Modification from the method : - Temperature: higher temperatur variation

Exposure : A pretest was carried out at 0,1 / 0,3 / 1,0 / 3,0 / 10 / 30 and 100 ppm (SOP: MFT 002 01).
For the main test the concentrations were selected according to the results of the pretest as: 0,32 / 0,56 / 1,00 / 1,78 and 3,2 ppm.

Results : The LC_{50} after 96 h was : 0,54 ppm (graphically determinated)

after	graphically determinated	calculated*	confidence limit
24	0,94	1,03	0,84 - 1,27
48	0,69	0,64	0,49 - 0,84
72	0,54	0,54	0,40 - 0,72
96	0,54	0,54	0,40 - 0,72

* Spearman-Kärber

All of the concentrations value presented in this charts are average concentrations, see Table 2.

The measured concentrations are presented in Table 2.

ECOLOGICAL 5

Bowman, J.H. *Acute Toxicity of D1063.01 (Triclosan; Irgasan® DP300) to Fathead Minnow (Pimephales promelas)*. ABC Laboratories Project Number 38655. July 20, 1990.

VOLUME 2 OF 3 OF SUBMISSION

STUDY TITLE

Acute Toxicity of D1063.01 (Triclosan; Irgasan® DP 300)
to Fathead Minnow (Pimephales promelas)

EPA GUIDELINE NO. 72-1

AUTHOR

Jane H. Bowman, Biologist II

STUDY COMPLETED ON: July 20,1990

CONDUCTED BY

Analytical Bio-Chemistry Laboratories, Inc.
Aquatic Toxicology Division
7200 East ABC Lane
P.O. Box 1097
Columbia, Missouri 65205

LABORATORY PROJECT IDENTIFICATION NO. 38655

PAGE 1 OF 34

SPONSOR

The Procter & Gamble Company
11810 East Miami River Road
Ross (Butler County), OH 45061

SUMMARY

The acute toxicity of D1063.01 to fathead minnow (*Pimephales promelas*) was assessed using the methods outlined by the protocol for this study. Water quality parameters of temperature, dissolved oxygen and pH were measured throughout the test and were within acceptable limits. Culture and acclimation records indicated the fish were in good condition for testing.

The study was conducted at the following nominal concentrations of D1063.01: 0.056, 0.10, 0.18, 0.32 and 0.56 mg/l. Ten fish, with a mean weight of 0.040 (±0.020) g and a mean standard length of 15 (±2) mm, were exposed to each test concentration and control.

The results of the four day static fish toxicity study using D1063.01 are summarized below. The 24-, 48- and 72-hour LC_{50} values were also determined.

Compound	96-Hour LC_{50} (95% C.I.)
D1063.01	0.26 mg/l (0.18 and 0.32 mg/l)

The slope of the 96-hour dose-response line was 14. Abnormal effects of mortality, loss of equilibrium, fish on the bottom of test chamber, quiescence, surfacing, labored respiration and/or erratic swimming were observed during the 96-hour exposure period in the 0.18 and 0.32 test concentrations. Complete mortality occurred in the 0.56 mg/l test chamber within 24 hours. One fish died in the solvent control chamber at 24 hours. This mortality was believed to be an aberration since all other fish in the solvent control chamber and all fish in the control chamber were normal throughout the 96 hour test period.

ECOLOGICAL 6

Pedersen, C.A. and Helsten, B.R. *Triclosan (Irgasan DP300®): 14-Day Acute Oral LD50 Study in Bobwhite Quail.* Bio-Life Associates, Ltd. Project Number 102-024-03. April 19, 1993.

VOLUME 3 OF 10 OF SUBMISSION

BIO-LIFE ASSOCIATES, LTD.
N6230 County Road G
Neillsville, Wisconsin 54456

AVIAN TOXICOLOGY
WILDLIFE STUDIES
FIELD INVESTIGATIONS
RESIDUES STUDIES
LARGE ANIMAL STUDIES

PHONE NO: 715-743-4557
FAX NO: 715-743-4109

STUDY TITLE

Triclosan (IRGASAN DP300®):
14-Day Acute Oral LD_{50} Study
in Bobwhite Quail

DATA REQUIREMENT

U.S. EPA FIFRA Guideline
Subdivision E, No. 71-1

AUTHORS

Carol A. Pedersen, B.S.
Bryan R. Helsten, B.S.

STUDY COMPLETION

April 19, 1993

PERFORMING LABORATORY

Bio-Life Associates, Ltd.
N6230 County Road G
Neillsville, WI 54456

LABORATORY PROJECT ID

BLAL No. 102-024-03

SPONSOR

CIBA-GEIGY Corporation
Chemicals Division
P.O. Box 18300
410 Swing Road
Greensboro, NC 27419

BIO LIFE

FINAL REPORT

I. SUMMARY

Pedersen, C.A. and B.R. Helsten (1993). Triclosan (IRGASAN DP300®): 14-Day Acute Oral LD_{50} Study in Bobwhite Quail, Bio-Life Associates, Ltd., BLAL # 102-024-03, issued April 19, 1993.

Bio-Life Associates, Ltd. conducted a 14-day acute oral LD_{50} study in bobwhite quail from September 15, 1992 to October 2, 1992 with Triclosan (IRGASAN DP300®) [Batch # 5.2.0211.0; 99.7%; CAS Reg. No. 3380-34-5] was administered on day 1, as a single oral dose via gelatin capsules, at levels of 0, 316, 464, 681, 1000, and 1470 mg/kg body weight to six groups of 21-week-old bobwhite quail (Colinus virginianus). All birds were observed on the day of dosing and for 13 consecutive days thereafter. Each group consisted of five male and five female birds. A Control group consisted of five male and five female birds and each bird in this group received one empty gelatin capsule.

The birds were purchased from Sand Prairie Quail Farm (Route 3 Box 159, Maquoketa, IA 52060). The quail were phenotypically indistinguishable from wild birds and were 15 weeks old when they were received at Bio-Life Associates, Ltd. All birds were from the same hatch (4/20/92 hatch date).

The birds dosed at 316 mg/kg body weight experienced a mortality on 9/16/92; therefore, a no-observed-effect level was not achieved in this study. At the request of the Sponsor, an additional Test group (T-I-A: 147 mg/kg) of ten birds (five male and five female) was dosed on 9/18/92. These birds were observed on the day of dosing and for 13 additional days thereafter.

The acute oral LD_{50} of Triclosan (IRGASAN DP300®) was determined to be 862 mg/kg body weight, with 95% confidence limits of 635 and 1170 mg/kg body weight. Since a small amount of diarrhea was noted under the 147 mg/kg pen on test day 6 only, a no-observed-effect level (NOEL) was not achieved in this study.

ECOLOGICAL 7

Pedersen, C.A. and Helsten, B.R. *Triclosan (Irgasan DP300®): 8-Day Acute Dietary LC50 Study in Bobwhite Quail.* Bio-Life Associates, Ltd. Project Number 102-022-01. April 19, 1993.

VOLUME 5 OF 10 OF SUBMISSION

BIO-LIFE ASSOCIATES, LTD.
N6230 County Road G
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AVIAN TOXICOLOGY
WILDLIFE STUDIES
FIELD INVESTIGATIONS
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PHONE NO: 715-743-4557
FAX NO: 715-743-4109

STUDY TITLE

Triclosan (IRGASAN DP300®):
8-Day Acute Dietary LC_{50} Study
in Bobwhite Quail

DATA REQUIREMENT

U.S. EPA FIFRA Guideline
Subdivision E, No. 71-2

AUTHORS

Carol A. Pedersen, B.S.
Bryan R. Helsten, B.S.

STUDY COMPLETION

April 19, 1993

PERFORMING LABORATORY

Bio-Life Associates, Ltd.
N6230 County Road G
Neillsville, WI 54456

LABORATORY PROJECT ID

BLAL No. 102-022-01

SPONSOR

CIBA-GEIGY Corporation
Chemicals Division
P.O. Box 18300
410 Swing Road
Greensboro, NC 27419

BIO LIFE

FINAL REPORT

I. SUMMARY

Pedersen, C.A. and B.R. Helsten (1993). Triclosan (IRGASAN DP300®): 8-Day Acute Dietary LC_{50} Study in Bobwhite Quail, Bio-Life Associates, Ltd., BLAL # 102-022-01, issued April 19, 1993.

Triclosan (IRGASAN DP300®) was tested in an 8-day acute dietary LC_{50} study conducted with bobwhite quail from December 12 to December 20, 1992. Triclosan (IRGASAN DP300®) [Batch # 5.2.0211.0; 99.7%; CAS Reg. No. 3380-34-5] was fed in the diet at levels of 312, 625, 1250, 2500, and 5000 ppm to five groups of bobwhite quail (*Colinus virginianus*) for a period of five days followed by a three-day recovery period. Each group consisted of ten 13-day-old bobwhite quail of indeterminate sex. Two similarly-sized Control groups received stock diet mixed with acetone. The birds were purchased from Stevenson Game Bird Farm (P.O. Box 426, Riverside, TX 77367). All birds were from the same hatch (11/29/92 hatch date). Five mortalities (one in the 2500 ppm Test group and four in the 5000 ppm Test group) were recorded throughout the investigation. The average body weight was depressed in the 5000 ppm Test group on test days 5 and 8. Reduced feed consumption was noted during the first five test days in the 5000 ppm Test group. The no-observed-effect

level (NOEL) was determined to be 1250 ppm. The 8-day acute dietary LC_{50} of Triclosan (IRGASAN DP300®) was determined to be greater than 5000 ppm.

ECOLOGICAL 8

Pedersen, C.A. and Helsten, B.R. *Triclosan (Irgasan DP300®): 14-Day Acute Oral LD50 Study in Mallard Ducks.* Bio-Life Associates, Ltd. Project Number 102-023-04. March 29, 1993.

VOLUME 4 OF 10 OF SUBMISSION

BIO-LIFE ASSOCIATES, LTD.
N6230 County Road G
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PHONE NO. 715-743-4557
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AVIAN TOXICOLOGY
WILDLIFE STUDIES
FIELD INVESTIGATIONS
RESIDUES STUDIES
LARGE ANIMAL STUDIES

STUDY TITLE

Triclosan (IRGASAN DP300®): 14-Day Acute Oral LD_{50} Study in Mallard Ducks

DATA REQUIREMENT

U.S. EPA FIFRA Guideline Subdivision E, No. 71-1

AUTHORS

Carol A. Pedersen, B.S.
Bryan R. Helsten, B.S.

STUDY COMPLETION

March 29, 1993

PERFORMING LABORATORY

Bio-Life Associates, Ltd.
N6230 County Road G
Neillsville, WI 54456

LABORATORY PROJECT ID

BLAL # 102-023-04

SPONSOR

CIBA-GEIGY Corporation
Chemicals Division
P.O Box 18300
410 Swing Road
Greensboro, NC 27419

Page 1 of 37

BIO LIFE

FINAL REPORT

I. SUMMARY

Pedersen, C.A. and B.R. Helsten (1993). Triclosan (IRGASAN DP300®): 14-day Acute Oral LD_{50} Study in Mallard Ducks, Bio-Life Associates, Ltd., BLAL # 102-023-04, issued March 29, 1993.

Triclosan (IRGASAN DP300®) was tested in a 14-day acute oral LD_{50} study in mallard ducks conducted November 17 to December 1, 1992. Triclosan (IRGASAN DP300®) [Batch # 5.2.0211.0; 99.7%; CAS No. 3380-34-5] was administered on day 1, as a single oral dose via gelatin capsules, at levels of 681, 1470, and 2150 mg/kg body weight to three groups of 19-week-old mallard ducks (*Anas platyrhynchos*). These birds were observed on the day of dosing and for 13 consecutive days thereafter. Each group consisted of five male and five female birds. A Control group consisted of five male and five female birds and each bird in this group received four empty gelatin capsules. The birds were purchased from Whistling Wings, Inc. (113 Washington Street, Hanover, IL 61041). The ducks were phenotypically indistinguishable from wild birds and were 15 weeks old when they were received at Bio-Life Associates, Ltd.

The no-observed-effect level (NOEL) was determined to be 2150 mg/kg body weight. The acute oral LD_{50} of Triclosan (IRGASAN DP300®) was determined to be greater than 2150 mg/kg body weight.

ECOLOGICAL 9

Hicks, S.L. and Stuerman, L.M. *Acute Toxicity of Triclosan to Scenedesmus subspicatus.* ABC Laboratories Study Number 43118. February 21, 1997.

Laboratories

received 3/19/97

Columbia, Missouri

SPONSOR

CIBA-GEIGY Corporation
410 Swing Road
Greensboro, North Carolina 27419

STUDY TITLE

Acute Toxicity of Triclosan to
Scenedesmus subspicatus

DATA REQUIREMENTS

U.S. EPA-TSCA, 40 CFR, Part 797
Guideline 797.1050

AUTHORS

Stephen L. Hicks
Scientist/Study Director

Luke M. Stuerman
Research Scientist

STUDY COMPLETION DATE

February 21, 1997

PERFORMING LABORATORY

ABC Laboratories, Inc.
Environmental Toxicology
7200 E. ABC Lane
Columbia, Missouri 65202

ABC LABORATORIES' STUDY NO.

Final Report #43118

Page 1 of 62

ABSTRACT

The primary objective of this test was to evaluate the acute toxicity of Triclosan to *Scenedesmus subspicatus* under static conditions following the procedures outlined in ABC Protocol No. TSCA 797.1050. The test was designed to yield EC_{50} values following 24, 48, 72, and 96 hours of exposure, if data permit. If possible, the 96-hour EC_{20} and EC_{80} value were to be calculated, and the 96-hour no-observed effect concentration (NOEC) was to be determined. The definitive study was conducted from May 6, 1996 to May 10, 1996.

The 96-hour acute toxicity test was conducted by exposing *Scenedesmus subspicatus* to the following nominal exposure concentrations: 0.50, 1.0, 2.0, 4.0, 8.0 µg/L Triclosan along with a control and vehicle blank (1.0 mL 10 mM NaOH/L). The test was conducted in 250-mL Erlenmeyer flasks containing 100 mL of the appropriate test solution. The 0.50, 2.0, 4.0, and 8.0 µg/L nominal exposure solutions and the control and vehicle blank were replicated five times (A,B,C,D, and E). The 1.0 µg/L nominal exposure solution was replicated nine times (A,B,C,D,E,F,G,H, and I). Each replicate received 1.0 mL of algal inoculum containing approximately 1.0×10^6 cells/mL, resulting in approximately 1.0×10^4 cells/mL for each flask. Water quality parameters of temperature and pH were measured at 0, 72, and 96 hours of the study. The temperature of the test solutions ranged from 23 to 25°C and the pH values ranged from 7.5 to 10.2.

Samples for analytical confirmation of Triclosan concentrations were collected at 0, 24, 48, 72, and 96 hours. All test levels were sampled at 0, 72, and 96 hours. Only the 1.0 µg/L test level was sampled at 24 and 48 hours. The overall mean measured concentrations of Triclosan (based on the time-weighted average of the 0, 72, and 96 hour sample points) were 0.36, 0.69, 1.5, 3.1, and 6.1 µg/L for the 0.50, 1.0, 2.0, 4.0, and 8.0 µg/L test levels, respectively. The overall mean measured test concentrations ranged from 69 to 78% of the nominal test concentrations. Characterization of the extracted radioactivity was performed on all sampled test levels at all sample points. Of the extracted radioactivity in the test levels, the percentage present as ^{14}C-Triclosan at 0 hour ranged from 94 to 100%. By 96 hours, the percentage present as ^{14}C-Triclosan ranged from 78 to 86%. For the fortification samples, the percentage present as ^{14}C-Triclosan ranged from 92 to 100% at 0 hour, and from 94 to 98% at 96 hours.

All results are based on biomass (i.e. cells/mL) at the end of the study. The 96-hour NOEC was determined to be 0.69 µg/L, since a significant difference ($p \leq 0.05$) for cell density was not shown for this exposure concentration as compared to the vehicle blank. The significant difference determined for the 0.36 µg/L exposure concentration was considered aberrant since the 0.69 µg/L exposure concentration was not shown to be significantly different. The 96-hour EC_{20}, EC_{50}, and EC_{80} values, based on cell density, were 1.2 µg/L (95% confidence limits = 0.81 and 1.5 µg/L), 1.4 µg/L (95% confidence limits = 1.4 and 1.5 µg/L), and 1.8 µg/L (95% confidence limits = 1.4 and 2.1 µg/L), respectively.

ABC LABS #43118-9

ECOLOGICAL 10

Drottar, K.R. and Krueger, H.O. *Triclosan: A 96-Hour Toxicity Test With the Freshwater Alga (Anabaena flos-aquae).* Wildlife International, Ltd. Project Number 473A-101. November 25, 1998.

TRICLOSAN:
A 96-HOUR TOXICITY TEST WITH THE
FRESHWATER ALGA (*Anabaena flos-aquae*)

FINAL REPORT

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER: 473A-101

U.S. Environmental Protection Agency
Series 850 - Ecological Effects Test Guidelines
OPPTS Number 850.5400

AUTHORS:

Kurt R. Drottar
Henry O. Krueger, Ph.D.

STUDY INITIATION DATE: October 21, 1997

STUDY COMPLETION DATE: November 25, 1998

Submitted to

Ciba Specialty Chemicals
4090 Premier Drive
High Point, North Carolina 27265

WILDLIFE INTERNATIONAL LTD.
8598 Commerce Drive
Easton, Maryland 21601
(410) 822-8600

SUMMARY

SPONSOR:	Ciba Specialty Chemicals
STUDY SPONSOR:	Dr. Per Stensby
TECHNICAL REPRESENTATIVE:	Dr. Donna J. D'Angelo
LOCATION OF STUDY, RAW DATA AND A COPY OF THE FINAL REPORT:	Wildlife International Ltd. Easton, MD 21601

WILDLIFE INTERNATIONAL LTD. PROJECT NUMBER:	473A-101
TEST SUBSTANCE:	Triclosan
STUDY:	Triclosan: A 96-Hour Toxicity Test with the Freshwater Alga (*Anabaena flos-aquae*)
MEAN MEASURED CONCENTRATIONS:	Negative Control, Solvent Control, 0.42, 0.81, 1.7, 3.4, 6.3 and 13 µg Triclosan/L
TEST DATES:	Experimental Start - January 19, 1998 Exposure Termination - January 23, 1998 Experimental Termination - February 1, 1998
LENGTH OF TEST:	96 Hours

TEST ORGANISM:	Freshwater Alga (*Anabaena flos-aquae*)
SOURCE OF TEST ORGANISMS:	Wildlife International Ltd. Easton, Maryland 21601

96-HOUR Ec10: 95% CONFIDENCE LIMITS:	0.97 µg Triclosan/L 0.85 and 0.99 µg Triclosan/L
96-HOUR Ec50: 95% CONFIDENCE LIMITS:	1.6 µg Triclosan/L 1.5 and 1.7 µg Triclosan/L
96-HOUR Ec90: 95% CONFIDENCE LIMITS:	5.6 µg Triclosan/L 4.8 and 5.8 µg Triclosan/L
STATISTICALLY-DEFINED NO OBSERVED EFFECT CONCENTRATION:	0.81 µg Triclosan/L

ECOLOGICAL 11

Wuthrich, V. *Acute Toxicity (LC50) Study of FAT 80'023/Q to Earthworms.* RCC Project Number 262956. July 5, 1990.

R C C

U M W E L T C H E M I E A G

EINGANG
FC 6.1
9. JULI 1990

Study Title:

ACUTE TOXICITY (LC50) STUDY OF FAT 80'023/Q TO EARTHWORMS

Data Requirement:

OECD No. 207, Section 2

Author:

Mrs. V. Wüthrich

Study Completion Date:

July 5, 1990

Performing Laboratory:

R C C UMWELTCHEMIE AG
P.O. Box
CH - 4452 Itingen/BL
Switzerland

Study Project No. :

RCC PROJECT 262956

Telephone: 061/98.52.52 / Telex: 966.136 RCC CH
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SUMMARY

The toxicity of FAT 80'023/Q to earthworms was tested in an artificial soil system according to the OECD Guideline 207: "Earthworm, Acute Toxicity Tests".

Based on mortality data, the test article proved to be non-toxic to earthworms up to a concentration of the test article in the soil (dry weight basis) of 1026 mg/kg (LC50: >1026 mg/kg).

The reference compound, 2-chloracetamide, showed an LC50 value of 55.63 and 75.19 mg per kg soil after 7 and 14 days of exposure.

ECOLOGICAL 12

Schwab, D. and Heim L.G. *FDA Seedling Growth Phytotoxicity Test.* ABC Laboratories Report Number 42620. April 7, 1997.

Laboratories

received 5/1/97 to Ciba

Columbia, Missouri

STUDY TITLE

FDA Seedling Growth Phytotoxicity Test

DATA REQUIREMENTS

FDA TAD-4.07

AUTHOR

Dave Schwab
Study Director/Research Scientist
Lucas G. Heim
Scientist

STUDY COMPLETED ON

April 7, 1997

PERFORMING LABORATORY

ABC Laboratories, Inc.
Analytical Chemistry and Field Studies
7200 E. ABC Lane
Columbia, Missouri 65202-8015

SUBMITTING LABORATORIES

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909 River Road
Piscataway, New Jersey 08855-1343
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ABSTRACT

A seed germination and seedling growth phytotoxicity study was conducted based on the FDA Technical Assistance Document 4.07 to define the range of toxicity of CP-001 to cucumber based on a no-observed effect concentration (NOEC). The NOEC is defined as the highest treatment concentration which is not significantly different from the controls. Radio-labeled concentrations of CP-001 were tested at nominal levels of 10, 30, 100, 300, and 1000 micrograms of CP-001 per kilogram of soil (μg/kg). A water control and vehicle blank control (acetone) were also included. The vehicle blank and CP-001 solutions for each treatment level were pipetted onto the soil in glass containers and mixed (homogenized) by strapping the glass containers onto a mixer.

Data collected in the seedling growth study included the visual phytotoxicity ratings, percent emergence, shoot lengths, dried shoot weights, and dried root weights. The data were analyzed using PC SAS statistical software. Analysis of variance at the alpha = 0.05 level of significance was used to determine the significant difference between the CP-001 treatments compared to the vehicle blank or pooled controls (vehicle blank and water control).

Visual ratings were compared to the vehicle blank, due to the acetone in the vehicle blank delaying germination slightly. The water control plants (shoots) were slightly larger than the other plants and this was attributed to quicker germination. By the end of the test (Day 28), the water control exhibited slight chlorosis in one replicate. The vehicle blank and 300 μg/kg treatments exhibited no phytotoxic symptoms. The 10 μg/kg treatment exhibited slight stunting in two replicates. The 30, 100, and 1000 μg/kg treatments exhibited slight stunting in only one replicate each. None of the visual ratings were considered significant.

Percent emergence showed no difference between the CP-001 treatments and the pooled controls, with percent emergence ranging from 96 to 100.

For shoot lengths, the plants in the water control were 16% longer than the plants in the vehicle blank (believed to be due to earlier germination in the water control). Therefore, the shoot lengths of the CP-001 treated cucumbers were compared to the lengths of the plants in the vehicle blank, as opposed to the pooled controls. CP-001 treatments were not significantly different than the vehicle blank.

Results of the cucumber shoot weight comparisons were similar to shoot length results. Once again, the comparison of the CP-001 treatments was made to the vehicle blank. This was due to the water control being 23% heavier than the vehicle blank, which was attributed to the water control germinating slightly quicker. CP-001 treatments were not significantly different than the vehicle blank.

Root weight showed a significance at alpha = 0.05 compared to the pooled controls. The significance was a positive increase (54%) at the 300 μg/kg level for root weight. While all the CP-001 treatments were heavier than the pooled controls (ranging from 30-54% larger), a dose response was not evident. The highest level (1000 μg/kg) did not show a significant increase in root weight. The increase in root weight at the 300 μg/kg concentration level was not

visually apparent, and it was not conclusive that the CP-001 caused this increase in root weight. Therefore, the NOEC was established at >1000 μg/kg.

The following table gives the NOEC for all the parameters tested in the study.

NOEC LEVELS FOR CUCUMBER

% EMERGENCE μg/kg	SHOOT LENGTH μg/kg	SHOOT WEIGHT μg/kg	ROOT WEIGHT μg/kg
>1000	>1000	>1000	>1000

The dosing solutions for each concentration were analyzed by liquid scintillation counting (LSC) prior to dosing of the soil. Following dosing, the purity of the dosing solutions was verified by high-performance liquid chromatography (HPLC). The dosing solution analysis confirmed stability of the test chemical throughout the dosing procedure.

At Day-0, duplicate soil samples for the blank, 30, 100, and 300 μg/kg treatment levels were analyzed by combustion radioanalysis followed by soil extraction and subsequent LSC and HPLC of each soil extract. For the 10 μg/kg and 1000 μg/kg concentrations, duplicate soil samples from the top, middle, and bottom of the dosing vessels were taken and analyzed to ensure homogeneity of the test chemical upon dosing. The test chemical was shown by both combustion radioanalysis and soil extract analysis to be homogenous throughout the soil sample.

At Day-14, 21, and 28 (termination), duplicate test sample soils for each concentration (blank, 10, 30, 100, 300, and 1000 μg/kg) were analyzed by combustion radioanalysis followed by soil extraction and subsequent LSC and HPLC analysis of each soil extract. The results of the Day-0, 14, 21, and 28 soil analysis are summarized as follows.

	Nominal	Mean ^{14}C-CP-001 Concentration (μg/kg)			
Treatment	Concentration	Day-0	Day-14	Day-21	Day-28
B	Blank	< LOQ	< LOQ	< LOQ	< LOQ
C	10 μg/kg	10.2 ± 0.5	3.94 ± 0.21	2.75 ± 0.26	2.07 ± 0.12
D	30 μg/kg	29.1 ± 1.6	11.8 ± 0.5	8.39 ± 0.10	7.24 ± 0.14
E	100 μg/kg	95.5 ± 1.1	36.9 ± 1.1	28.6 ± 0.6	20.5 ± 0.5
F	300 μg/kg	299 ± 6	136 ± 1	90.6 ± 4.0	67.5 ± 1.5
G	1000 μg/kg	913 ± 22	392 ± 7	290 ± 7	191 ± 6

ECOLOGICAL 13

Hoberg, J.R. *(D1063)- Determination of Effects on Seedling Growth of Six Plant Species.* Springborn Laboratories, Inc. Report Number 90-12-3574. June 23, 1992.

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