

"Contains NO CBI"

# Monsanto

ENVIRONMENT, SAFETY & HEALTH

Monsanto Company  
800 N. Lindbergh Boulevard  
St. Louis, Missouri 63167  
Phone: (314) 694-1000

November 25, 1992

Non-Confidential

Registered Mail  
Return Receipt Requested  
Triplicate Copies Enclosed

Document Processing Center (TS-790)  
Office of Toxic Substances  
Environmental Protection Agency  
401 M Street, SW  
Washington, DC 20460

Attention: Section 8(e) Coordinator (CAP Agreement)

This submission is pursuant to the TSCA Section 8(e) Compliance Audit Program and CAP Agreement #8ECAP-0036. This information was inadvertently overlooked as we were assembling our final submission under CAP. This study has been added to the Monsanto final report for the CAP.

The information included is characterized as follows:

Chemical Identity: Santonox R; Santowhite Crystals: 4,4'-thiobis(3-methyl-6-t-butyl)phenol  
Chemical CAS No.: 96695

Information/Study Type: (II,B,2,b)/Acute, Environmental

Title: Acute Toxicity of Santowhite Crystals to Fathead Minnows: A Time Independent Study  
Study Identification #: MO-92-9051

Summary of reportable adverse effects: Submitted due to a high order of toxicity in an aquatic organism.

It should be noted that environmental effects were previously reported for this material under the CAP, as shown on Appendix A.

It should be noted that this summary may not highlight all adverse effects that EPA may judge to meet TSCA 8(e) reportability.

No information in this submission is trade secret or confidential business information.

Sincerely,

J.R. Condray  
Director, Regulatory Management  
(314) 694-8833

(A)

8E HQ-92-12061  
INIT  
88920010300

92 DEC -2 AM 10:35  
OFS DOCUMENT RECEIPT OF C

RECEIVED  
12/7/94

ACUTE TOXICITY OF SANTOWHITE CRYSTALS® TO FATHEAD MINNOWS  
A TIME INDEPENDENT STUDY

### INTRODUCTION

As part of the aquatic assessment plan for Santowhite Crystals, a time independent toxicity test was conducted. This test allows for the calculation of a more accurate LC50 value than a static acute test, is run for a longer period of time and provides an indication of the potential for the chemical to be an accumulative toxin. Chemicals which are accumulatively toxic demonstrate increasingly lower LC50 values over time. Such substances generally have the greatest potential to induce sublethal chronic effects at low exposure concentrations over long periods of time.

### CONCLUSIONS

The toxicity of Santowhite Crystals to fathead minnows was assessed in a 14-day flow-through toxicity test. The LC50 values ranged from 0.21 mg/l on day 1 to 0.054 mg/l on day 14. These data suggest this chemical is an accumulative toxin and is highly toxic to fathead minnows under the conditions of this test.

### BIOLOGICAL

The fathead minnows (Pimephales promelas) used in the test were obtained from EG&G Bionomics, Wareham, Massachusetts. All test fish were held in culture tanks at 18°C and observed for at least fourteen days prior to testing (Appendix I). During this period, the fish received a standard fish food (Purina No. 3). Throughout the study, the fish were fed once a day. Mortality during the fourteen days prior to the initiation of the test was less than 1% indicating that the fish were in good condition. The fish for this experiment had a mean (S.D.) weight of 0.39 (0.26) gm and mean length of 34.5 (6.9) mm.

### EXPOSURE SYSTEM

A continuous flow diluter system, developed at the Monsanto Aquatic Laboratory (previously described in ES-78-SS-21) was used to deliver water and Santowhite Crystals for six test concentrations and a solvent control (Figure 1). Dimethylformamide (DMF) was used as a carrier solvent. Maximum concentration of DMF was 0.33 ml/l. Six stock solutions were pumped at a rate of 2 ml/hour through a 0.8 mm I.D. x 1.5 mm O.D. Teflon® line to just beneath the surface of the water in each of six 250 ml mixing cells. Water entered each cell at the rate of 6.0 l/hour. The water and Santowhite Crystals were mixed by magnetic stirrers placed under each cell. The water flowed directly from the mixing cells to the test tanks. Both the water and Santowhite Crystals were injected continuously. The following nominal exposure concentrations were chosen for this study: 0.04, 0.08, 0.16, 0.25, 0.4 and 0.6 mg/l. These concentrations were based on data from a static acute 96-hour toxicity study. The 96-hour LC50 was 0.36 mg/l.

The test tanks were all glass 17-liter aquaria with test volume of 15 liters. A flow rate of 6 l/hour provided a sufficient amount of water for six tank

volume replacements per day. The tanks were kept at ambient room temperature. The mean and range of temperature, dissolved oxygen, alkalinity and hardness were 22.2 (22-23)°C, 7.2 (6.8-7.5) mg/l, 313 (312-314) mg/l, and 292 (290-294) mg/l, respectively. The pH ranged from 7.6 to 8.0. Additional water quality characteristics are presented in Table 2 and Appendix I. The dilution water used for this study was well water known to be of high quality.

The test was initiated on 8/20/79 by random assignment of 30 fathead minnows to each of the six test aquaria. The test chemical was flowing through the system for at least 24 hours prior to introducing the fish. Mortality and behavioral observations were made once every 24 hours for the duration of the study. Dead individuals, if present, were removed at each observation. LC50 values were calculated according to the methodology of Litchfield and Wilcoxon (1949). The protocols for this study were the Environmental Sciences time independent protocol (Adams et al, 1979, ES-79-M-1) and the EPA Committee on Methods for Toxicity Tests with Aquatic Organisms (1975).

### TEST COMPOUND

A one-half pint sample in a sealed glass bottle was received from John Vander Kooi (Akron, Ohio) on 4/17/78. The sample was labeled with the following number: N 109-006. Upon receipt, the sample was observed to be in good condition and was noted to be white crystals. The sample was stored in the dark at room temperature. A master stock of 18 g/l was prepared on 8/14/79 and used to make all subsequent substocks for the duration of the study.

### RESIDUE ANALYSIS

The actual concentrations of Santowhite Crystals in the test aquaria were determined seven times during the study period. Samples, dates and regimen are shown in Table 3. Samples were analyzed by collecting 50 mls of water from the test aquaria and extracting it once with 3 mls of nanograde hexane. The extracts were analyzed by gas chromatography using a HP 5710A gas chromatograph (G-C). The GC operating conditions were: temperature, isothermal at 250°C; column, 1 meter-3 mm I.D.-OV101; attenuation, 8x10; and detector, flame ionization. The extraction efficiency was calculated by determining the percent recovery of Santowhite Crystals from test water. Duplicate fifty milliliter samples of test water were spiked to give nominal concentrations of 1.92 mg/l and 0.08 mg/l. Each sample was extracted once with 2 mls of hexane and analyzed. The average percent recoveries for the 0.08 and the 1.92 mg/l samples were 87.7 and 98.0 percent, respectively. The water analyses reported in eable 3 are not corrected for percent recovery. All LC50 values were calculated based on measured exposure concentrations.

### STUDY DEVIATIONS

The following deviations from the time independent protocol (ES-79-M-1) are noted (1). The study was initially started on 8-20-79 with five test concentrations and a solvent control. An additional test concentration of 0.031 mg/l was started on 8-22-79. This was the lowest concentration tested and added to insure that a concentration was tested which did not kill 50 percent of the fish. Only one fish died in the 0.031 mg/l test level. The raw data sheets reflect observations and analytical analysis on day 16, however, the fish at each test level were exposed for only 14 days. The day 16 observation reflects

the 2-day lag for the 0.031 mg/l test level. (2) Water samples were not collected from test aquaria when 100 percent of the fish were dead. Therefore, only two sets of samples were analyzed in the two highest exposure concentrations. And (3) an additional water sample was analyzed for the 0.066 mg/l test concentration on 9/4/79 to further document the exposure level in this test aquaria. The diluter system was not turned off until day 14 (9/5/79) for the 0.031 mg/l exposure level. These deviations are not judged to invalidate or affect the study results.

## RESULTS

The mean (+ 1 standard deviation) Santowhite Crystal concentration in each test aquaria is shown in Table 3. The exposure concentrations were somewhat variable, but judged to be satisfactory.

The study was terminated after 14 days. The LC50 values ranged from 0.21 mg/l (day 1) to 0.054 mg/l (day 14, Table 1). Mortality data is shown in Table 4. Santowhite Crystals judged to be an accumulative toxin based on the decrease in the LC50 value throughout the 14-day exposure period (Figure 2). The data (Figure 2) suggests that little or no additional mortality would have occurred if the test had continued. No abnormal behavioral symptoms or syndromes were noted during the study. On the basis of this test, Santowhite Crystals should be considered highly toxic to fathead minnows.

Figure 1. Continuous flow chemical dilution system.

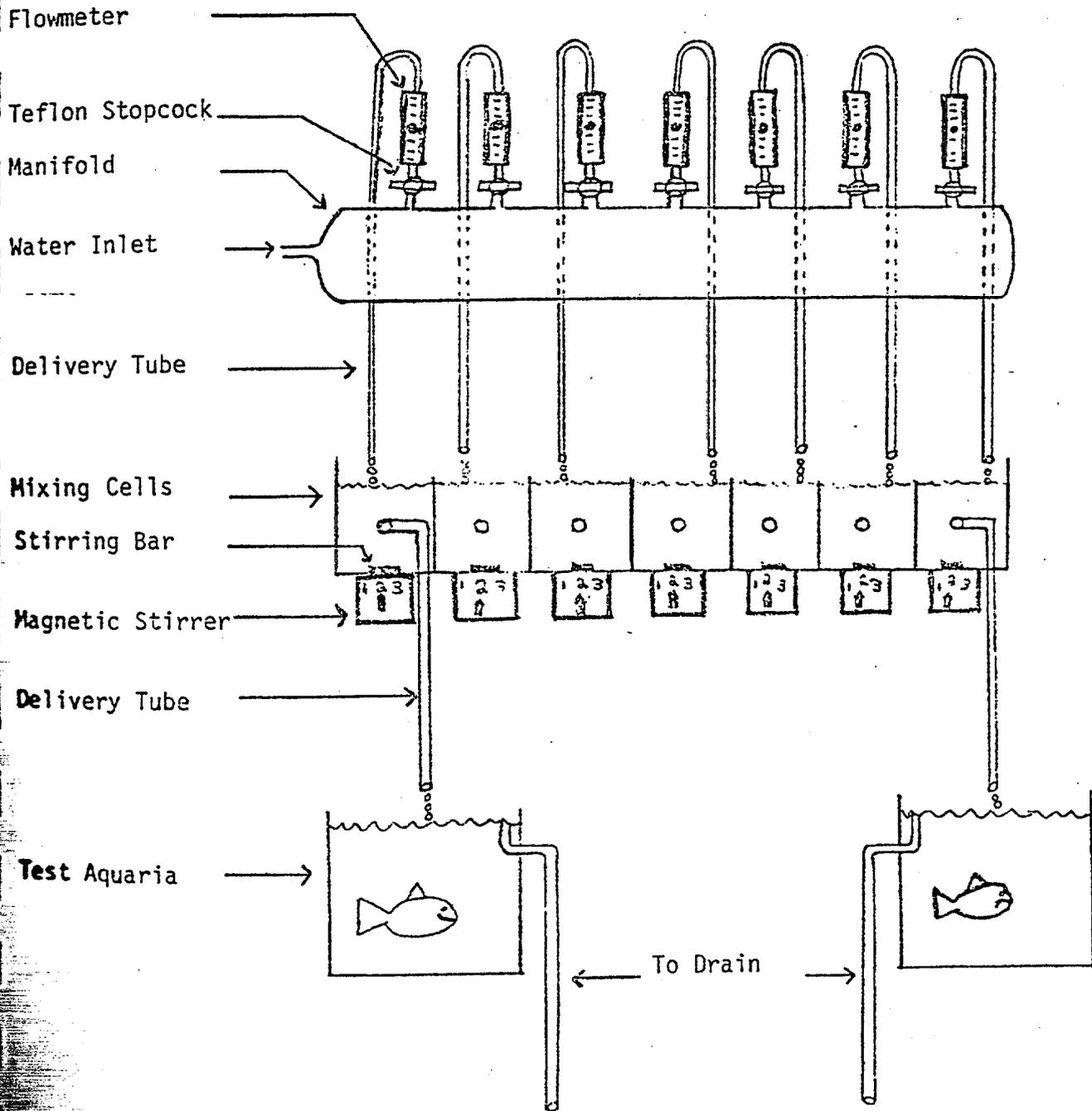


TABLE 1. LC50 values determined during a 15-day flow-through toxicity test with Santowhite Crystals and fathead minnows. The values are based on mean measured concentrations.

Days of Exposure	LC50 (95% C.I.) (mg/l)
1	0.21*
2	0.17 (0.15-0.19)
3	0.15 (0.12-0.20)
4	0.14 (0.11-0.17)
7	0.080 (0.064-0.099)
14	0.054 (0.046-0.063)

\*Confidence intervals are not shown because the line drawn through the mortality data was not a good fit by the Chi squared test method and therefore the LC50 value must be considered as an estimate.

TABLE 2. Average water quality characteristics of the dilution (well) water.

Characteristic	Well Water Measurement
Alkalinity (mg/l CaCO <sub>5</sub> )	303
Hardness (mg/l CaCO <sub>5</sub> )	297
pH (median)	8.10
Aluminum (mg/l Al)	0.014
Ammonia-total (mg/l N)	0.35
Ammonia-unionized (mg/l NH <sub>4</sub> )	0.001
Antimony (mg/l Sb)	0.006
Barium (mg/l B)	<0.035
Beryllium (mg/l Be)	0.001
Cadmium (mg/l Cd)	<0.001)
Calcium (mg/l Ca)	67.0
Chloride (mg/l Cl <sup>-</sup> )	54.5
Chromium (mg/l Cr)	<0.009
Cobalt (mg/l Co)	0.002
Copper (mg/l Co)	0.005
Fluoride (mg/l F <sup>-</sup> )	1.90
Iron (mg/l Fe)	0.013
Lead (mg/l Pb)	0.01
Magnesium (mg/l Mg)	2.79
Manganese (mg/l Mn)	0.001
Molybdenum (mg/l Mo)	0.005
Nickel (mg/l Ni)	0.025
Phosphorus (mg/l P)	0.006
Silicon (mg/l Si)	4.3

TABLE 2. (Continued)

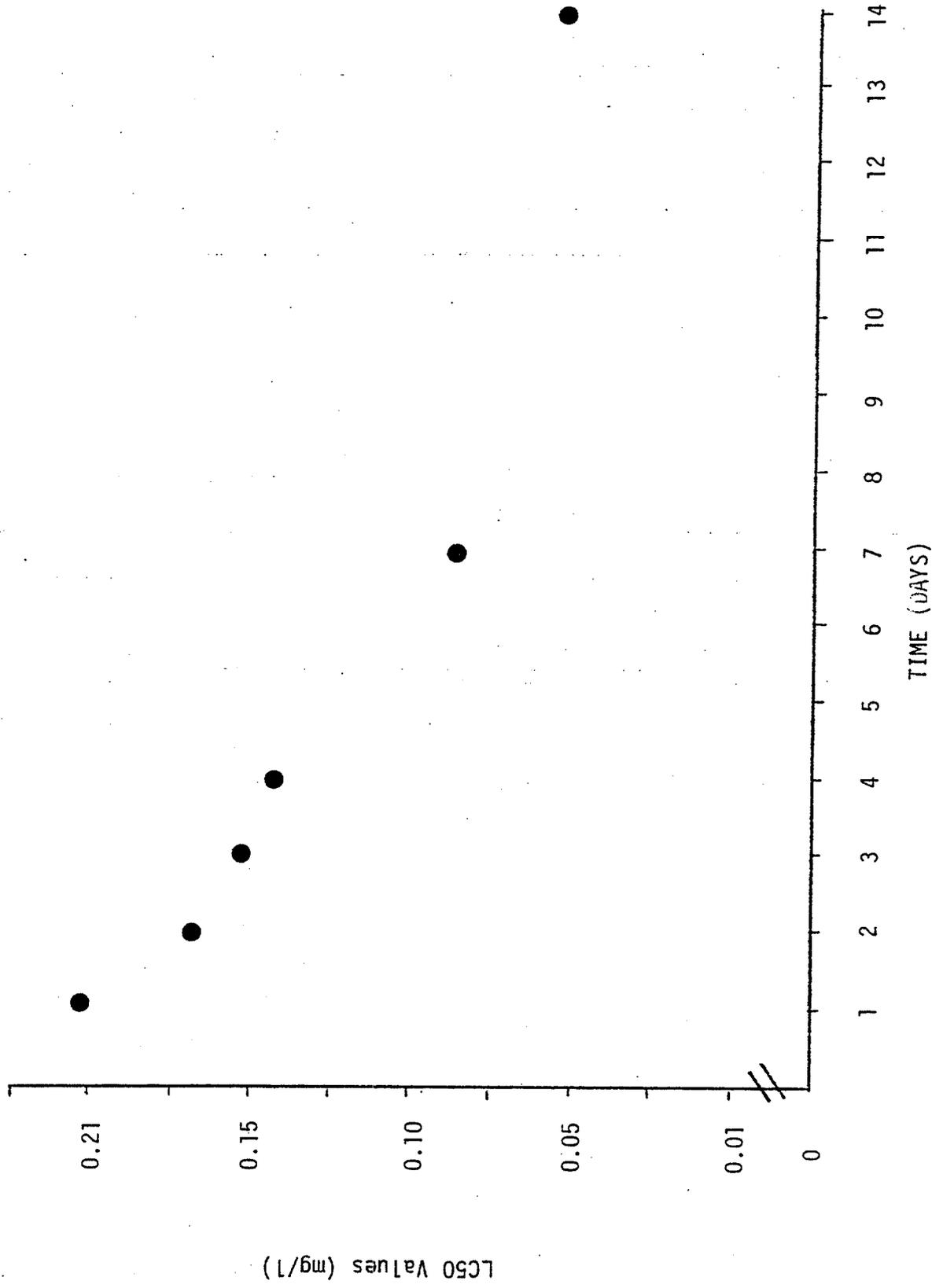
Characteristic	Well Water Measurement
Silver (mg/l Ag)	<0.001
Sodium (mg/l Na)	83.2
Sulfate (mg/l SO <sub>4</sub> <sup>-</sup> )	175.4
Strontium (mg/l Sr)	1.30
Tin (mg/l Sn)	0.006
Titanium (mg/l Ti)	0.001
Total Organochlorine (ug/l)	<0.5
Total Organophosphorus (ug/l)	<0.05
Vanadium (mg/l V)	0.14
Zinc (mg/l Zn)	0.001

TABLE 3. Measured and nominal concentrations of Santowhite Crystals during a 14-day toxicity test.

Nominal Concentrations (mg/l)	Mean Concentration														
	Day 0	Day 1	Day 2	Day 4	Day 8	Day 11	Day 15	Day 16	(+1 S.D.)						
Solvent Control	-	<0.002	<0.002	<0.002	<0.002	-	<0.002	-	<0.002	-	<0.002	-	<0.002	-	<0.002
0.04	-	-	-	-	0.058	0.022	-	0.014	-	0.031	0.023	-	0.031	0.023	0.031 (0.023)
0.08	0.040	0.057	0.077	0.105	-	0.040	0.015	-	0.066	0.025	-	0.066	0.025	0.066 (0.025)	
0.16	0.11	0.20	0.20	-	0.18	-	-	-	0.17	0.04	-	0.17	0.04	0.17 (0.04)	
0.25	0.19	0.32	0.33	-	-	-	-	-	0.28	0.08	-	0.28	0.08	0.28 (0.08)	
0.40	0.25	0.40	-	-	-	-	-	-	0.32	-	-	0.32	-	0.32	
0.60	0.58	0.75	-	-	-	-	-	-	0.67	-	-	0.67	-	0.67	



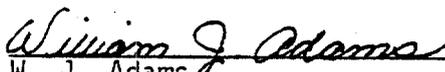
FIGURE 2. Acute toxicity curve for Santowhite Crystals with fathead minnows. Measured concentrations were used to calculate LC50 values.



Submitted by:

Monsanto Industrial Chemicals Company  
Environmental Sciences Section  
Aquatic Toxicology Laboratory - N1B  
800 North Lindbergh Blvd.  
St. Louis, Missouri 63166

Prepared by:

  
\_\_\_\_\_  
W. J. Adams  
Senior Research Biologist

Approved by:

  
\_\_\_\_\_  
W. E. Gledhill  
Research Group Leader

LITERATURE CITED

1. Adams, W.J., W. J. Renaudette and W. E. Gledhill. 1978. Acute Toxicity of Santicizer S-160 to Fathead Minnows in a Flow-Through System. Environmental Sciences Special Study ES-78-SS-21.
2. Adams, W.J., B. B. Heidolph and W. E. Gledhill. 1979. Methodology for Conducting Time Independent Flow-Through Toxicity Studies with Fish. Environmental Sciences Special Study ES-79-M-1.
3. Committee on Methods for Toxicity Tests with Aquatic Organisms. 1975. Methods of Acute Toxicity Tests with Fish, Macroinvertebrates and amphibians. C. E. Stephens, Chairman. U.S. EPA. Ecol.Res.Ser. 660/3-7509.
4. Litchfield, J.T., Jr. and F. Wilcoxon. 1949. A Simplified Method of Evaluating Dose-Effect Experiments. J.Pharm.& Exp.Ther. 96: 99-113.

## APPENDIX A

Previous environmental effect submissions under #8ECAP-0036 for Santonox R, Santowhite Crystals, CAS #96695.

Acute Toxicity of Santowhite Crystals to Daphnia (AB780354)  
Acute Toxicity of Santowhite Crystals to Fatheads (AB790209)



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460

J. R. Condray  
Director, Regulatory Management  
Monsanto Agricultural Company  
800 North Lindbergh Boulevard  
St. Louis, Missouri 63167

OFFICE OF  
PREVENTION, PESTICIDES AND  
TOXIC SUBSTANCES

FEB 27 1995

EPA acknowledges the receipt of information submitted by your organization under Section 8(e) of the Toxic Substances Control Act (TSCA). For your reference, copies of the first page(s) of your submission(s) are enclosed and display the TSCA §8(e) Document Control Number (e.g., 8EHQ-00-0000) assigned by EPA to your submission(s). Please cite the assigned 8(e) number when submitting follow-up or supplemental information and refer to the reverse side of this page for "EPA Information Requests".

All TSCA 8(e) submissions are placed in the public files unless confidentiality is claimed according to the procedures outlined in Part X of EPA's TSCA §8(e) policy statement (43 FR 11110, March 16, 1978). Confidential submissions received pursuant to the TSCA §8(e) Compliance Audit Program (CAP) should already contain information supporting confidentiality claims. This information is required and should be submitted if not done so previously. To substantiate claims, submit responses to the questions in the enclosure "Support Information for Confidentiality Claims". This same enclosure is used to support confidentiality claims for non-CAP submissions.

Please address any further correspondence with the Agency related to this TSCA 8(e) submission to:

Document Processing Center (7407)  
Attn: TSCA Section 8(e) Coordinator  
Office of Pollution Prevention and Toxics  
U.S. Environmental Protection Agency  
Washington, D.C. 20460-0001

EPA looks forward to continued cooperation with your organization in its ongoing efforts to evaluate and manage potential risks posed by chemicals to health and the environment.

Sincerely,

*Terry R. O'Bryan*  
Terry R. O'Bryan  
Risk Analysis Branch

Enclosure

12061A



Recycled/Recyclable  
Printed with Soy/Canola Ink on paper that  
contains at least 50% recycled fiber

**Triage of 8(e) Submissions**

Date sent to triage: MAR 08 1995

NON-CAP

CAP

Submission number: 12061A

TSCA Inventory:

Y

N

D

Study type (circle appropriate):

Group 1 - Dick Clements (1 copy total)

ECO

AQUATO

Group 2 - Ernie Falke (1 copy total)

ATOX

SBTOX

SEN

w/NEUR

Group 3 - Elizabeth Margosches (1 copy each)

STOX

CTOX

EPI

RTOX

GTOX

STOX/ONCO

CTOX/ONCO

IMMUNO

CYTO

NEUR

Other (FATE, EXPO, MET, etc.): \_\_\_\_\_

Notes:

**THIS IS THE ORIGINAL 8(e) SUBMISSION; PLEASE REFILE AFTER TRIAGE DATABASE ENTRY**

**For Contractor Use Only**

entire document: 0 1 2 pages 1 pages 1, tab

Notes:

Contractor reviewer: LPS Date: 1/25/95

CECATS TRIAGE TRACKING DBASE ENTRY FORM

CECATS DATA: Submission # 8EHO-1292-12061 SEQ. A

TYPE: INT. SUPP FLWP

SUBMITTER NAME: Monsanto Company

SUB. DATE: 1/25/92

12/02/92

CSRAD DATE: 12/07/94

CHEMICAL NAME: Santonox R

Santo white Crystals

CAS# 96-69-5

" "

INFORMATION REQUESTED: FLWP DATE:  
 0501 NO INFO REQUESTED  
 0502 INFO REQUESTED (TECH)  
 0503 INFO REQUESTED (VOL ACTIONS)  
 0504 INFO REQUESTED (REPORTING RATIONALE)  
 DISPOSITION:  
 0630 REFER TO CHEMICAL SCREENING  
 0678 CAP NOTICE

VOLUNTARY ACTIONS:  
 0401 NO ACTION REPORTED  
 0402 STUDIES PLANNED/IN PROGRESS  
 0403 NOTIFICATION OF WORKING RESUMES  
 0404 LABELS/MSDS CHANGES  
 0405 PROCESS/AND/OR CHANGES  
 0406 APPAUSE DISCONTINUED  
 0407 PRODUCTION DISCONTINUED  
 0408 CONFIDENTIAL

INFORMATION TYPE:	L F C	INFORMATION TYPE:	L F C	INFORMATION TYPE:	L F C	INFORMATION TYPE:	L F C
0201 ONCO (HUMAN)	01 02 04	0216 EPICLIN	01 02 04	0241 IMMUNO (ANIMAL)	01 02 04	0277 ALLERG (HUMAN)	01 02 04
0202 ONCO (ANIMAL)	01 02 04	0217 HUMAN EXPOS (PROD CONTAM)	01 02 04	0242 IMMUNO (HUMAN)	01 02 04	0278 ALLERG (ANIMAL)	01 02 04
0203 CELL TRANS (IN VITRO)	01 02 04	0218 HUMAN EXPOS (ACCIDENTAL)	01 02 04	0243 CHEM/PHYS PROP	01 02 04	0279 METAB/PHARMACO (ANIMAL)	01 02 04
0204 MUTA (IN VITRO)	01 02 04	0219 HUMAN EXPOS (MONITORING)	01 02 04	0244 CLASTO (IN VITRO)	01 02 04	0280 METAB/PHARMACO (HUMAN)	01 02 04
0205 MUTA (IN VIVO)	01 02 04	0220 ECO/AQUA TOX	01 02 04	0245 CLASTO (ANIMAL)	01 02 04	0281 CHRONIC TOX (ANIMAL)	01 02 04
0206 REPRO/TERATO (HUMAN)	01 02 04	0221 ENV. OCCUR/REL FATE	01 02 04	0246 CLASTO (HUMAN)	01 02 04	0282 CHRONIC TOX (ANIMAL)	01 02 04
0207 REPRO/TERATO (ANIMAL)	01 02 04	0222 EMER INCI OF ENV CONTAM	01 02 04	0247 DNA DAM/REPAIR	01 02 04	0283 CHRONIC TOX (HUMAN)	01 02 04
0208 NEURO (HUMAN)	01 02 04	0223 RESPONSE REQ/EST DELAY	01 02 04	0248 PROD/USE/PROC	01 02 04	0284 CHRONIC TOX (ANIMAL)	01 02 04
0209 NEURO (ANIMAL)	01 02 04	0224 PRODCOMP/CHEM ID	01 02 04	0251 MSDS	01 02 04		
0210 ACUTE TOX. (HUMAN)	01 02 04	0225 REPORTING RATIONALE	01 02 04	0259 OTHER	01 02 04		
0211 CHR. TOX. (HUMAN)	01 02 04	0226 CONFIDENTIAL	01 02 04				
0212 ACUTE TOX. (ANIMAL)	01 02 04	0227 ALLERG (HUMAN)	01 02 04				
0213 SUB ACUTE TOX (ANIMAL)	01 02 04	0228 ALLERG (ANIMAL)	01 02 04				
0214 SUB CHRONIC TOX (ANIMAL)	01 02 04	0229 METAB/PHARMACO (ANIMAL)	01 02 04				
0215 CHRONIC TOX (ANIMAL)	01 02 04	0230 METAB/PHARMACO (HUMAN)	01 02 04				

USE: \_\_\_\_\_ PRODUCTION: \_\_\_\_\_

TOXICOLOGICAL CONCERN:

LOW  
MED  
HIGH

SPECIES

Fish  
Daphnia

ONGOING REVIEW

YES (DROP/REFER)  
NO (CONTINUE)  
LEFT-R

NON-CBI INVENTORY

YES  
NO

CAS SR

IN HUMAN

1-25-92