

US EPA ARCHIVE DOCUMENT

ENVIRONMENTAL FATE AND GROUND WATER BRANCH**Review Action**

To: W. Waldrop/A. Ertman
Product Manager 71, Registration Division (H7505C)

From: Akiva Abramovitch, Section Head, Environmental Fate Review Section #3
Environmental Fate and Ground Water Branch
Environmental Fate and Effects Division (H7507C) *AK*

Thru: Hank Jacoby, Chief, Environmental Fate and Ground Water Branch
Environmental Fate and Effects Division (H7507C) *Henry Jacoby 4/5/94*

Attached, please find the EFGWB review of...

changed to 4/5/94 (etc 5/14/94)

DPBarcode:	D194459, D199452, D199841, D199871		
Common Name:	Chlorothalonil	Trade name:	Bravo
Company Name:	ISK Biotech (formerly Fermenta, Diamond Shamrock, etc.)		
ID #:	050534-00008		
Purpose:	submission of data on oyster bioaccumulation, various administrative actions		

Type Product:	Action Code:	EFGWB #(s):	Review Time:
fungicide	various	n.a.	

STATUS OF STUDIES IN THIS PACKAGE:

Guideline #	MRID	Status ¹
162-1	000873-51	C
165-4	various*	A
165-5 ¹	430706-01	A

STATUS OF DATA REQUIREMENTS:

	Status ²
161-1	S
161-2	P
161-3	P
162-1	P
162-3	S
162-4	S
163-1	S
164-1	U
165-1	U
165-2	U
165-4	S

* MRID#s 000866-29, 000294-11, and 000866-30 comprise the complete study.

¹Study Status Codes:

A=Acceptable U=Upgradeable C=Ancillary I=Invalid.

²Data Requirement Status Codes:

S=Satisfied P=Partially satisfied N=Not satisfied R=Reserved.

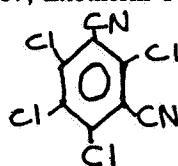
1

This study was required by EEB, but is being reviewed by EFGWB per their request.

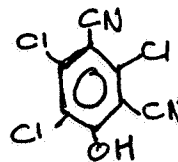


1. CHEMICAL:

chemical name: 2,4,5,6-tetrachloro-1,3-benzenedicarbonitrile
common name: Chlorothalonil
trade name: Bravo, Clortosip, Daconil 3787, Exotherm Termil.
structure:
CAS #:
Shaughnessy #: 081901



Chlorothalonil



SDS-3701

2. TEST MATERIAL: see DER

3. STUDY/ACTION TYPE:

D194459 -- discussion of previously submitted field dissipation data

D199452 -- data on bioaccumulation in oysters

D199841 -- discussion of aerobic soil metabolism, bioaccumulation in fish, half-life and general properties -- NO NEW DATA

D199871 -- meeting follow-up -- NO NEW DATA

4. STUDY IDENTIFICATION:

Kabler, K. and Quinn, B. Chlorothalonil: Bioconcentration Test with the Eastern Oyster *Crassostrea virginica*, under Flow-Through Conditions. sponsored and submitted by ISK Biotech Corporation, Mentor, OH. performed by Toxikon Environmental Sciences, Jupiter, FL. dated 11/10/93; received by EPA 1/3/94 under MRID# 430706-01.

5. REVIEWED BY:

Typed Name: E. Brinson Conerly-Perks
Title: Chemist, Review Section 3
Organization: EFGWB/EFED/OPP

E.B. Conerly-Perks
3/25/94

6. APPROVED BY:

Typed Name: Akiva Abramovitch
Title: Section Head, Review Section 3
Organization: EFGWB/EFED/OPP

Akiva Abramovitch
APR 4 1994

7. CONCLUSIONS:

DP Barcode D194459 -- field dissipation of SDS-3701 -- EFGWB cannot completely accept the applicant's view as presented in this submission, although it appears to be qualitatively valid -- i.e., SDS-3701 can be seen to decrease by the end of the experimental period. EFGWB recognizes that the applicant's interpretation is not inconsistent with the data as presented, but it is not the only possible one.² The body of information on file indicates a certain amount of

² These data are not new, but are being used and described in a different way in the current submission.

persistence and mobility of SDS-3701. A precise $t_{1/2}$ has not been defined for SDS-3701, since no separate metabolism study has been done on this compound. In the current submission, the applicant uses selected data to contend that SDS-3701 is approximately as persistent as parent, i.e., has a similar $t_{1/2}$ range (6 to 43 days vs 8 to 36 days for parent *per the applicant*). However, if all data are considered, the concentration of SDS-3701 appears to peak between 31 and 60 days following a single application of chlorothalonil, and declines within the next 30 days to between 50 and 80% of its peak value (Appendix A, Table A.1, attached). Therefore the effective $t_{1/2}$ could be said to range between 30 and 75 days overall. Another way of describing the data is that ca. 5 to 20% of applied material is present as SDS-3701 ninety days after application (*ibid.*, 90-day column). For environmental fate evaluation, the most significant message in the data is that considerable amounts of SDS-3701 are present 3 months following an application of chlorothalonil. Although the field dissipation studies show it to be at least as mobile as parent, a precise depth of movement has not been defined. Therefore, SDS-3701 must be considered a potential leacher in some soils and sometimes persistent.

Chlorothalonil has an extensive history of data submissions. Although many studies on field dissipation have been submitted, none has been fully acceptable. The flaws were major, including failure to sample to sufficient depth. EFGWB has excused the applicant from any further investigation of field dissipation of chlorothalonil and its degradates, despite the lack of any fully acceptable studies. Rather than requiring additional studies, EFGWB has elected to take a "weight of evidence" approach based on the studies already in hand. This means that chlorothalonil is being considered persistent in some soils, for purposes of EFGWB assessments, with a $t_{1/2}$ as long as 60 days. We note that in the current submission the data for soil 1, silty clay loam, bears this out (Appendix A, Table A.1). It is also clear from the field dissipation studies that parent is potentially mobile in some soils. SDS 3701, as noted above, is also potentially mobile in some soils and sometimes persistent.

DP Barcode D199452 -- data on bioaccumulation in oysters

The study is acceptable to meet the requirement for data on bioaccumulation in bivalves. Although residues accumulate (BCF ca. 2600x), they are not parent chlorothalonil. They do not readily depurate (25% remains after 14 days). The apparent reason for this is that the residues derive from metabolic fragments of chlorothalonil which have entered the "carbon pool" and become integrated into the biochemical cycles of the oysters. 50 to 75% of the ^{14}C was non-extractable even using conditions that digested the tissue. Only a fraction (34 - 52%) of the tissue residue was eliminated during depuration, but this can be attributed to the non-extractable nature of the residue. Thus, chlorothalonil itself did not bioconcentrate in oysters and the accumulated ^{14}C represented polar metabolites and bound residues.

DP Barcode D199841 -- discussion of aerobic soil metabolism, bioaccumulation in fish, half-life and general properties

aerobic soil metabolism -- The relevant study, MRID# 000873-51, was initially accepted in 1988, but upon reexamination was deemed only partially acceptable (EBC 7/12/89). The applicant believes that this data requirement should be considered fulfilled for the reasons stated below. EFGWB does not agree. The major reason for rejection was the large proportion of material which was bound and not further identified. Since metabolism is the key route of transformation and disappearance of chlorothalonil, EFGWB believes that the applicant and the environment will be better served by submission of a new and more definitive study rather than continuing to attempt to repair this one. This is especially true in light of the marginal nature of the field dissipation information. The applicant's reasoning, together with EFGWB's response follows:

- 1) The available information established that the primary mechanism of degradation is microbial.
EFGWB agrees.
- 2) Under aerobic conditions in the laboratory, the $t_{1/2}$ for chlorothalonil is 10.3 to 36.5 days.
EFGWB agrees.
- 3) The soil degrade SDS-3701 is not persistent in soil...[but]...has half life values ($t_{1/2}$) under laboratory conditions ranging from 6-43 days based on the original soil data generated in four soils.
EFGWB does not necessarily agree (see above discussion re field dissipation) with the half-life figure or the classification. The data have at least one alternative interpretation. Even if the figure of 43 days is a real upper limit, EFGWB considers that to indicate at least a moderate degree of persistence.

- 4) The aged leaching study provides adequate accountability of radiolabelled material in that study. *EFGWB agrees that it is acceptable for that study, but that study provides only a single point of analysis. Although it may have certain elements in common with the soil metabolism study, the data from it cannot be directly incorporated to repair the metabolism study.*
- 5) [Paraphrased] Although the extraction in the metabolism study was done under mild conditions, it fulfilled the requirements of EFGWB. The materials which were classified as "bound" following this mild extraction are considered by EFGWB to be unavailable for uptake and/or movement through soil. *Although current policy does not require the use of destructive methods of extraction, a single extraction under relatively mild conditions does not necessarily serve to demonstrate unextractability or unavailability to biota in the environment.*

fish bioaccumulation -- The applicant believes that the data provided in MRID#s 000866-29, 000294-11, and 000866-30 taken together should be acceptable to fulfill the requirement for this guideline. The studies themselves have been reexamined for this review. This reexamination reinforced EFGWB's judgement that this set of studies is not acceptable, although the difficulties of testing the compound are recognized. The data clearly indicate that uptake of some labelled material does occur, and that depuration is relatively slow.

EFGWB rejected these studies in a review dated 11/29/89 for these two principal reasons:

- 1) The concentration of chlorothalonil was as low as 0.0004 ppm vs a nominal 0.008 and an observed 0.0059 total ¹⁴C. The point of this comment was that the concentration of chlorothalonil was low relative to both the nominal concentration and the measured concentration of radiolabelled material in the water.

The applicant does not challenge the accuracy of EFGWB's understanding of the data, but responds that the nominal concentration was chosen to meet the guidelines for toxicity. EFGWB grants that the nominal concentration was properly chosen.

The response does not answer the intent of the EFGWB comment, which is discussed in (2) below.

- 2) The fish were exposed to a constantly changing and undefined mixture of compounds.

The applicant responds that the transformations are unavoidable and are due to bacterial metabolism taking place in the water. They cite as evidence an accepted aquatic metabolism study, and state that in a "real life" exposure situation metabolism would rapidly remove chlorothalonil.

EFGWB notes that in the currently submitted oyster study, the chlorothalonil concentration was apparently maintained at ca. 85% of its nominal value, despite the obvious technical difficulties. EFGWB also notes that in the aquatic metabolism study the initial rapid disappearance of parent may have been due to binding by the sediment, rather than to metabolic transformation. The fish study did not use sediment.

In the case of the fish bioaccumulation study, the cause of the observed rapid transformation has not been clearly defined. There are at least two possibilities:

- 1) bacterial metabolism -- If bacteria were responsible for the transformation, and if the bacterial content of the water in the fish study was comparable to that of the metabolism study, it could explain the observed results. However, the study would probably have been initiated with a relatively low concentration of bacteria. The source of much of the bacterial activity in the aquatic metabolism study would have been sediment, which was not present in the fish study. Moreover, without sediment and with constant replenishment of aquarium water, bacterial activity may never have become sufficiently high to produce the effects which were reported. This possibility is neither proved nor disproved.

- 2) metabolism by the fish -- In fact, the original report of the fish study mentions the fish as a possible agent of metabolism. However, ideally, this type of study should be done such that the population of fish is low enough not to materially affect the concentration of pesticide; if the fish do significantly metabolize the pesticide, then in most cases the study should be rejected on those grounds. Lacking a "no fish" control, it is impossible to establish whether the fish were in fact having such an effect. Once again, there is no direct evidence which proves or disproves this theory.

In a study such as this, results are difficult or impossible to interpret if the material to which the test organism is exposed is not well defined. This is definitely the case here. In a situation such as a spill into a body of water, fish could be exposed to a significant concentration of parent chlorothalonil for at least a short period of time, and it is this extreme or rare case which, among others, this study guideline is intended to address. It may not be possible to avoid all degradation of chlorothalonil while performing the study. However, if the parent pesticide is added rapidly enough relative to the "residence time" of the compound (taking into account the possible effects both of bacteria and fish), then the mix in the exposure tank should be relatively high in parent, and low in degradates. This would allow assessment of the uptake potential of parent compound.³

DP Barcode D199871 -- meeting follow-up -- NO NEW DATA -- administrative material only -- no response required

8. RECOMMENDATIONS:

D194459 -- discussion of previously submitted field dissipation data -- the applicant should be informed that EFGWB has not changed the assessment of SD-3701. EFGWB considers it at least moderately persistent and potentially mobile.

D199452 -- data on bioaccumulation in oysters -- the applicant should be informed that the requirement for data on non-target species bioaccumulation (oysters) has been fulfilled. No further data are required at this time.

D199841 -- discussion of aerobic soil metabolism; fish bioaccumulation; half-life; general properties -- NO NEW DATA

aerobic soil metabolism -- The deficiencies in the previously submitted study have not been repaired. The applicant should be informed that this data requirement is still not fulfilled, and that a new study is required. EFGWB is concerned with the high percentage of material considered "bound" after only a single attempt at extraction under mild conditions. It is possible, although not certain, that there may be unidentified degradates of toxicological significance. Although destructive techniques of extraction are not required or even encouraged, EFGWB strongly recommends that several attempts be made before declaring the remaining material unextractable.

bioaccumulation in fish -- The deficiencies in the previously submitted study have not been repaired. The applicant should be informed that this data requirement is still not fulfilled, and that a new study is required. The study should be focussed on exposure to parent. It should be noted that although conditions in the oyster study were much less favorable, a high proportion of parent was maintained in that study.

D199871 -- meeting follow-up -- NO NEW DATA -- No response is necessary.

³ The average "residence time" is determined by the volume of the tank and the rate of addition of new material. The description of experimental conditions does not give this value, nor does it provide enough information that EFGWB can calculate it with confidence.

9. BACKGROUND:

EXECUTIVE SUMMARY

Available data, some not fully acceptable, indicate that chlorothalonil can be moderately persistent and is potentially mobile on terrestrial sites; a major degradate, SDS-3701 is also potentially persistent and mobile. The major pathway of disappearance is microbial metabolism. Chlorothalonil is widely used, particularly on peanuts.

ENVIRONMENTAL FATE ASSESSMENT

Some important fate data are partially acceptable or of marginal quality, but do allow some conclusions to be drawn.

The weight of evidence suggests that chlorothalonil is moderately persistent under some conditions when applied to terrestrial sites. It is susceptible to aerobic and anaerobic soil metabolism, but resistant to other processes. In an acceptable study, it proved stable to hydrolysis at pH 5 and 7; only 10% degraded in 30 days at pH 9. Acceptable aqueous photolysis and soil photolysis studies also indicate stability for at least 30 days. A partially acceptable soil metabolism study indicates a half-life of 10.3 to 36.5 days in various soils, but much of the radiolabelled material which remained after incubation was not characterized. Numerous partially acceptable and unacceptable terrestrial field dissipation studies indicate persistence under some conditions; $t_{1/2}$ s ranged from 14 - 59 days.

Chlorothalonil may be less persistent under flooded or aquatic conditions, due to apparently higher rates of metabolism by microbes. An acceptable anaerobic aquatic metabolism study supplied the required anaerobic soil metabolism data, indicating a 5-15 day half-life. Aerobic aquatic metabolism occurred with an apparent half-life of 1.4 hours; adsorption to sediment may have been the actual reason for the initial rapid disappearance. A run-off study is currently under review by the surface water group and a small-scale monitoring study protocol is under review by the ground-water group. These may serve to clarify both the mobility and persistence issues.

Although much of the evidence is marginal, the overall indication is that chlorothalonil is at least moderately mobile in some soils. Ground water monitoring studies have been required because of findings of a degradate (SDS-46851) in ground water. Laboratory adsorption/desorption studies indicate moderate mobility in sand ($K_d = 3$) to low mobility in silt ($K_d = 29$). In a leaching study done on sandy loam soil, parent compound did not leach, but a degradate (SDS 46851 @ 5.5% of applied) did. Partially acceptable and unacceptable field dissipation studies suggest that chlorothalonil and degradates could leach under appropriate conditions. These reports regularly include detections of parent in the lowest depths tested, and some findings of degradates.

An unacceptable fish bioaccumulation study reported BCF values of 264, 76 and 514 for whole fish, edible tissue, and viscera, respectively. These values are not insignificant, but do not meet the 1000x "trigger" value which would indicate the need for concern. In this study residues were quantitated but not further characterized. Depuration was ca. 75% complete in 14 days after exposure stopped. Because of widespread use, in particular the use on peanuts, certain ecotox triggers have been met. EEB has required estuarine studies, including the oyster bioaccumulation study reviewed in this document. This study on bioaccumulation of oysters in natural waters, showed that residues were incorporated and not easily depurated. This apparently resulted from rapid metabolism of chlorothalonil in the exposure water, and its entry into the "carbon pool". The observations from both studies may possibly reflect similar processes.

GROUND WATER ASSESSMENT

Because of its apparent potential for persistence and mobility as delineated above, chlorothalonil has the characteristics which indicate a possible threat to ground water in some situations. [*However, solubility in water is said to be low (0.6 ppm, Farm Chemicals Handbook, 1993).*] A monitoring study has been required because of residue findings; a protocol has been submitted and is under review.

SURFACE WATER ASSESSMENT

In a run-off event, chlorothalonil might reach surface water associated either with soil particles or dissolved in water. Under most conditions, chlorothalonil would be transformed by metabolism, $t_{1/2}$ of 5-15 days under anaerobic conditions or ca. 2 hours aerobically.

DATA BASE ASSESSMENT The data requirements are summarized below:

Hydrolysis -- fulfilled -- MRID # 00405-39 -- stable at pH 5 and 7; 10% degrades in 30 days at pH 9; 2,4,5,6-tetra-Cl-isophthalimide the only degradate

Aqueous Photolysis -- fulfilled -- MRID#s 000872-81, 401834-18, 000405-40, 1988 Reg. Std. -- MRID# 401834-18 indicates stability to photolysis in unsensitized water

Soil Photolysis -- fulfilled -- MRID# 001437-51, 1988 Reg. Std. -- indicates stability to photolysis on soil

Aerobic Soil Metabolism -- **partially fulfilled** -- The study which was discussed in the 1988 Registration Standard, MRID# 000873-51, is not adequate. An acceptable study (per Guidelines subpart N) must establish patterns of disappearance of parent; appearance and disappearance of degradates, and identity of degradates, and that study does not do so adequately. Although extraction methods were not exhaustive (employing only a single extraction with 4:1 acetone/0.3N HCl for 30 minutes), compounds representing from 40 to 75% of applied material were classified as "unextractable", and neither identified or quantified. These uncharacterized residues may represent compounds of toxicological concern.

Anaerobic Soil Metabolism -- fulfilled by acceptable anaerobic aqueous metabolism study (10/23/85, also HLB 4/22/86, MRID# 0014790-75)

Anaerobic Aquatic Metabolism -- fulfilled -- (10/23/85, HLB 4/22/86, MRID# 001479-75) -- $t_{1/2}$ 5-15 days; no significant volatile degradates were detected. Identified degradates were 4-hydroxy-2,5,6-trichloroisophthalonitrile, 3-cyano-2,4,5,6-tetrachlorobenzamide, 2-hydroxy-5-cyano-3,4,6-trichlorobenzamide, and 3-carboxy-2,5,6-trichlorobenzamide.

Aerobic aquatic metabolism -- fulfilled, (MRID# 422261-01, 2/3/93) -- Chlorothalonil dissipated rapidly (first $t_{1/2}$ = ca. 1 - 2 hr); none of the identified degradates appeared to persist. During the first few hours after dosing, soil bound material increased to a maximum of ca. 58% at 6 hours, and, after that, approximately 30-35% of applied material was bound to soil throughout the experimental period. The semilog plot of time vs concentration was not linear, and the mechanisms which were occurring are not clear; i.e. binding to the sediment may have been the first effect observed.

Leaching/Adsorption/Desorption -- fulfilled (8/1/86, MRID#s 001151-05, -001537-10) -- low leachability in lab for both aged and unaged material, but findings of residues in ground water triggered monitoring requirements (see attached ground water review). K_{ads} 3 (sand) to 29 (silt) in batch studies.

Terrestrial Field Dissipation -- **partially fulfilled** -- numerous unacceptable or partially acceptable studies strongly indicate a level of persistence and mobility which is grounds for concern. An attachment summarizes selected relevant data.

Laboratory Accumulation - Fish -- MRID#s 000866-29, 000294-11, 000866-30 have been cited, and are discussed in this document. These data are partially acceptable; they show accumulation (BCFs up to 500x) and slow depuration (75% complete at 14 days).

Laboratory Accumulation -- Oysters -- MRID# 430706-01, discussed in this review -- The observed BCF is ca. 2600x. Labelled residues, but not chlorothalonil itself, are found, and do not readily depurate. The apparent reason for this is that the residues derive from metabolic fragments of chlorothalonil which have entered the "carbon pool" and become integrated into the biochemical cycles of the oysters. 50 to 75% of the ^{14}C was non-extractable even using conditions that digested the tissue. Only a fraction (34 - 52%) of the tissue residue was eliminated during depuration but this can be attributed to the non-extractable nature of the residue. Thus, there was no bioconcentration of chlorothalonil in oysters and the accumulated ^{14}C represented polar metabolites and bound residues.

10. DISCUSSION OF INDIVIDUAL TESTS OR STUDIES: attached
11. COMPLETION OF ONE-LINER: new information added
12. CBI APPENDIX: attached to DER



TERRESTRIAL FIELD DISSIPATION DATA

Some of the studies which have been reviewed are summarized below. Also, a considerable number of studies were reviewed in detail in EBC 3/5/91. MRID # 000872-96 was reviewed in the 1988 Reg. Std. The following are the most recent.

MRID# 424338-13 -- The study is of marginal quality and is only partially acceptable to fulfill the data requirement. Although the study was not done under ideal conditions, it indicates a certain persistence and mobility of chlorothalonil. Because the soil was subjected to cultivation and harvest during the course of the study (apparently in an effort to combine features of both field dissipation and rotational crop investigations), it is difficult to interpret the results to obtain precise depth of half-life values and leaching capability. Chlorothalonil had an apparent field half-life of ca. 2 months. Also, the study does establish that under cultivation conditions the compound easily reached a depth of 9 inches, although the level to which chlorothalonil is likely to leach and remain detectable was not well defined. There were some detections in the lowest depth sampled, and a considerable number in the depth just above that. Moreover, SDS-3701 appeared to have slightly greater mobility than parent.

MRID# 415648-29 -- Fresno, CA. This study is unacceptable for several reasons. These data do not serve to define a pattern or time course for the dissipation of chlorothalonil under field conditions. Soil sampling may not have gone deep enough to define the extent of leaching of chlorothalonil and its degradates. Analyses were done on composited samples (and sampling variation thereby minimized), and therefore, EFGWB cannot assess the "inherent" precision and accuracy of the procedures. The study author reported a half-life of 58 days for chlorothalonil in the upper 12 inches of soil using *selected* data, but since values were arbitrarily discarded, the calculated half-life is considered to be of questionable validity.

MRID# 451648-28

At both treated plots, chlorothalonil residues were detected in the 12- to 15-inch depth, the lowest soil depth sampled. The soil should have been sampled to depths (preferably two sampling depths) at which residues were nondetectable. From selected data, the study author calculated a half-life of 40 days for chlorothalonil. The arbitrary exclusion of data used to calculate the half-life causes the resulting half-life to be of questionable value.

Donalsonville, GA.

The study is unacceptable. The data do not serve to define a pattern or time-course for the dissipation of chlorothalonil under field conditions because they are too randomly variable. Soil was only sampled through day 29 following the tenth application, except for samples taken at 222 days posttreatment. The depth of soil sampling was insufficient to define the extent of leaching of chlorothalonil and its degradates.

Chlorothalonil was detected to a depth of 12-inches. It dissipated with an observed half-life of 14-29 days from the upper 12 inches of a plot of sandy loam soil. The degradates were 4-hydroxy-2,5,6-trichloroisophthalonitrile(SDS-3701), 2-hydroxy-5-cyano-3,4,6-trichlorobenzamide(SDS-47525), 3-carboxy-2,5,6-trichlorobenzamide (SDS-46851), 3-cyano-2,4,5-trichlorobenzamide (SDS-47523/SDS-47524), 3-cyano-2,4,5,6-tetrachlorobenzamide (SDS-19221). The manufacturing impurities HCB and PCNB were isolated as deep as the 9- to 12-inch depth. *Per the authors* PCNB levels were related to the level of chlorothalonil residues present, but the levels of HCB were not. In addition, pretreatment samples taken from the two treated plots contained detectable residues of HCB (0.003-0.006 ppm).

Greenfield, CA.

The study is unacceptable. The cultural practices employed may have compromised study results. It is highly probable that chlorothalonil residues were transferred to lower soil depths. Accordingly, the concentration of pesticide may have been diluted by bringing pesticide-free soil from lower horizons, and may have increased the rate of dissipation by aerating the soil and presenting new nutrient sources to the microbial population. Also, samples may have been contaminated by the sampling process itself. Moreover, soil was not sampled deeply enough to define the extent of leaching of chlorothalonil and its degradates.

CHLOROTHALONIL

Page _____ is not included in this copy.

Pages 10 through 14 are not included in this copy.

The material not included contains the following type of information:

- _____ Identity of product inert ingredients.
 - _____ Identity of product impurities.
 - _____ Description of the product manufacturing process.
 - _____ Description of quality control procedures.
 - _____ Identity of the source of product ingredients.
 - _____ Sales or other commercial/financial information.
 - _____ A draft product label.
 - _____ The product confidential statement of formula.
 - _____ Information about a pending registration action.
 - ☒ FIFRA registration data.
 - _____ The document is a duplicate of page(s) _____.
 - _____ The document is not responsive to the request.
-

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

DATA EVALUATION REVIEW 1

I. Study Type: bioaccumulation, non-target organisms, guideline 72-6³

II. Citation:

Kabler, K. and Quinn, B. Chlorothalonil: Bioconcentration Test with the Eastern Oyster *Crassostrea virginica*, under Flow-Through Conditions. sponsored and submitted by ISK Biotech Corporation, Mentor, OH. performed by Toxikon Environmental Sciences, Jupiter, FL. dated 11/10/93; received by EPA 1/3/94 under MRID# 430706-01.

III. Reviewer:

Typed Name: E. Brinson Conerly-Perks
Title: Chemist, Review Section 3
Organization: EFGWB/EFED/OPP

E.B. Conerly-Perks
3/25/94

IV. Conclusions:

The study is acceptable to fulfill the requirement for bioaccumulation data on bivalves. The data indicate that residues, but not parent chlorothalonil, are found, and do not readily depurate. The apparent reason for this is that the residues derive from metabolic fragments of chlorothalonil which have entered the "carbon pool" and become integrated into the biochemical cycles of the oysters.

V. Materials and Methods:

ABSTRACT

A bioconcentration study was conducted with ¹⁴C-chlorothalonil in the Eastern Oyster, *Crassostrea virginica*, under flow-through conditions. The study was conducted with a twenty-five day exposure period at a target concentration of 0.7 µg/L chlorothalonil followed by a fourteen day depuration period.

The bioconcentration factor (BCF, calculated as ¹⁴C-concentration in tissue divided by mean ¹⁴C-concentration in water) for the day 21 and 25 samplings was 2660X. The decline in ¹⁴C tissue levels during the depuration period corresponded to a decrease of 34 to 52% from the levels in the last five days of the exposure period.

Analyses of the ¹⁴C-residues in tissue showed that no ¹⁴C-chlorothalonil was present. The ¹⁴C in tissue was comprised of polar metabolites and bound non-extractable residues.

Chlorothalonil was rapidly metabolized in the aquarium water. During the test, approximately ten water changes per day were employed to maintain the majority of ¹⁴C in the tank as chlorothalonil. A preliminary study demonstrated the rapid metabolism of chlorothalonil with almost 80% of the parent degraded in one hour.

VI. Study Author's Results and/or Conclusions:

RESULTS AS DESCRIBED BY THE STUDY AUTHOR

It was necessary to terminate the uptake portion of the study after 25 days instead of the scheduled 28 days because of high mortality in both the exposed and control oyster populations. This was discussed with the sponsor representative, and it was decided to terminate the uptake phase in order to maintain enough oysters for the depuration phase. A summary of the mortality of both the exposed and control oyster groups is presented in Table 2 (attached). Mortality exceeded recommended limits during the study, but it is believed that the mortality was associated with the heavy bacterial growth attributed to the co-solvent. This bacterial growth probably produced a microenvironment around each oyster subjecting the oysters to reduced water quality and may have affected feeding behavior. This occurred in spite

³ EFGWB is reviewing this study at the request of EEB who imposed the data requirement.



of efforts to avoid those conditions. As stated previously, oysters were removed from each tank on a daily basis to clean bacterial growth off the oysters. In addition, a series of preliminary studies were conducted to try to find an alternate solvent system and to minimize the solvent concentrations. Solubility and stability problems eliminated the use of other routinely utilized solvents (e.g., triethylene glycol and acetone) and necessitated the use of at least 1% ethanol as a co-solvent in water for stock preparation.

After 25 days of exposure, the concentration of ^{14}C (calculated as chlorothalonil equivalents) in the oyster tissue was determined to be $1.54 \mu\text{g/g}$ (Table 3 and Figure 3). The bioconcentration factor calculated for this sampling time was 1822 based on the mean water concentration of 0.845 ng/ml at day 25. However, since this was not the highest ^{14}C -residue level, the bioconcentration factor (BCF) was also calculated using the highest tissue concentration (day 21). Based on the 21 day tissue and mean water concentrations, the bioconcentration factor (BCF) was calculated to be 3498X. The highest ^{14}C -concentration in tissue, found in the day 21 sample, occurred in an oyster with the lowest tissue weight. For the day 21 and day 25 sampling times, the total dpm per oyster were comparable with $8.15 \times 10^6 \text{ dpm}$ for day 21 and $8.49 \times 10^6 \text{ dpm}$ for day 25 (calculated as $7.65 \times 1,065,448 \text{ dpm/g}$ and $11.0 \text{ g} \times 771,440 \text{ dpm/g}$, respectively). Based on total ^{14}C in tissue, it appears that a plateau for uptake was reached in the day 21 and 25 time frame. The average bioconcentration factor calculated using the results from the day 21 and 25 samplings was 2660X. The concentration of ^{14}C in the oyster tissue after 14 days of depuration was determined to be $1.02 \mu\text{g/g}$ and represented a 34% decrease in residue concentration during the depuration period from the day 25 result but was a decrease of 52% from the residue level observed for day 21.

In order to extract and characterize the ^{14}C -residues bound in the tissue of the exposed oysters, several solvent systems were evaluated. The original solvent system, acetone:0.3N HCl (8:2 v/v) was employed to extract spiked parent material, however, this method gave low extractability of ^{14}C -activity with samples from the treatment tank. A 10% aqueous solution of perchloric acid, as well as a concentrated solution of dimethylsulfoxide (DMSO) were also tried, however, the ^{14}C -recoveries were low (25-30% of theoretical). These data are presented in Table 4. In order to completely solubilize the oyster tissue and release any identifiable radioactivity that was present, concentrated phenol was added and the tissue was allowed to "digest" for approximately 24 hours to determine releasable radioactivity. As shown in Table 4 even using these extremely strong conditions for extraction only ≈ 25 to 50% of the total ^{14}C was extractable from day 21 or later. Aliquots of the phenol extracts from the day 7, 21, and 25 uptake phase and day 14 of the depuration phase, were analyzed by HPLC and TLC to characterize the extractable ^{14}C -residues. The results of these analyses (Figures 4 to 6) demonstrate that the degradates are more polar than chlorothalonil (bound to the origin on silica gel TLC) and do not match the identity of any of the available metabolites. No parent ^{14}C -chlorothalonil was observed in any of these phenol extracts. Therefore, there was no bioaccumulation of chlorothalonil in oysters and the accumulated ^{14}C represented polar metabolites and bound residues.

During the uptake phase the salinity of the dilution water ranged from 14 to 32‰ in the exposure treatment tank and 12 to 32‰ in the control tank (Table 5). The salinity excursion which occurred during the first week of the uptake phase resulted from an abnormally heavy rainfall event. During the depuration phase, the salinity of the dilution water ranged from 26 to 31‰ in the exposure tank and 28 to 31‰ in the control tank. The water temperature during the entire study ranged from 18.5 to 24°C in the exposure tank and 18.1 to 22.7°C in the control tank (Table 6). Dissolved oxygen concentrations during the uptake phase ranged from 4.2 to 7.9 mg/L in the exposure tank and 4.1 to 8.1 mg/L in the control treatment tank (Table 7). The accumulation of algae and bacterial slime in the tanks and on the oysters is believed to have contributed to the decrease in dissolved oxygen concentrations. During the depuration phase, the dissolved oxygen concentration of the dilution water ranged from 5.0 to 7.3 mg/L in the exposure tank and 5.4 to 7.3 mg/L in the control tank. The daily pH of the dilution water in both tanks ranged from 7.5 to 8.1 for the entire study (Table 8).

CONCLUSIONS

The results of this study indicate that accumulation of ^{14}C -residues (BCF=2660X) occurs in the tissues of the marine bivalve mollusc during continued exposure to chlorothalonil. Biodegradation of chlorothalonil in the exposure tank was minimized by maintaining rapid flow-through conditions with approximately ten (10) water changes per day. The assays of aquarium water during the study, which showed that at least 85% of the ^{14}C was chlorothalonil at each sampling, are



in contrast to the preliminary study where nearly 80% of applied chlorothalonil was metabolized in a natural water/sediment system within one hour. Therefore, it can be reasonably concluded that the exposure to chlorothalonil achieved in this laboratory study would not occur in the environment.

Analysis of oyster tissue showed that no chlorothalonil was present and 50 to 75% of the ^{14}C was non-extractable even using conditions that digested the tissue. Only a fraction (34 - 52%) of the tissue residue was eliminated during depuration but this can be attributed to the non-extractable nature of the residue. Thus, there was no bioconcentration of chlorothalonil in oysters and the accumulated ^{14}C represented polar metabolites and bound residues.

VII. Reviewer's Comments:

- 1) The study is acceptable to fulfill the requirement for data on bioaccumulation in bivalves. The data appear to indicate that chlorothalonil *per se* does not accumulate in oysters. It appears, instead, that chlorothalonil is quickly metabolized in natural waters, and that residues then enter the "carbon pool", from which they do become incorporated into the oysters.
- 2) As the applicant points out, the conditions of this investigation bear little resemblance to most levels of exposure which might occur in nature, but force at least an "extreme worst case" or "worse than worst case" situation. In addition, it is not clear to this reviewer whether the frequent handling of the animals might have in itself had adverse effects on them, resulting in the "excessive mortality".

VIII. CBI Information Addendum: attached



CHLOROTHALONIL

Page _____ is not included in this copy.

Pages 18 through 33 are not included in this copy.

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