TOX review of chlorothalonil

Mr. R. Coberly, Biologist TOX Branch

Mr. E. Wilson (PM #21)

Pesticide Petition No: 6F1799

Petitioner: Diamond Shamrock

Request: Establish a tolerance of 0.2 ppm in or on soybeans for 2,4,5,6-tetrachloroisophthalonitrile and its metabolite 4-hydroxy-2,5,6-trichloroisophthalonitrile

Recommendation: Do not establish tolerance because of the following two reasons:

1. The allowable daily intake has been reached.
2. An 18 month mice oncogenicity study has not been provided.

Comments: Due to the numerous inadequacies in the three generation rat reproduction testing of the 4-hydroxy metabolite, the study is judged not to fulfill regulatory requirements. This decision does not affect the request for a tolerance on the parent chemical at this point in time. Also, the rat chronic feeding study on the 4-hydroxy metabolite does not satisfy the oncogenic requirement because the length of the study was less than 24 months.

Related Petitions: 7G0516, 7F0599, 9F0743, 1F1024, 2F1230, 3F1362, 4F1502, 5E1569, 6F1749, 6E1761, 6H5136, 6G1813, and 6E1841.

Existing Tolerances: $180.275

15 ppm in or on celery

5 ppm in or on broccoli, brussels sprouts, cabbage, cauliflower, cucumbers, melons, onions (green), pumpkins, snap beans, squash (summer and winter) and tomatoes

3.0 ppm in or on passion fruit

1.0 ppm in or on carrots and sweet corn (kernels plus cob with husks removed)

0.5 ppm in or on onions (dry bulb)

0.3 ppm in or on peanuts

0.1 ppm in or on potatoes
Chemical Name: 2,4,5,6-tetrachloroisophthalonitrile

Synonyms: Daconil, DAC-2787, Forturf

Structure:

Use: Fungicide

Formulation: Bravo 6F EPA #677-313

Active Ingredient

54.00% chlorothalonil

Inert Ingredients

*cleared under 40 CFR 180.1001(c)

INERT INGREDIENT INFORMATION IS NOT INCLUDED
Background Information

Following is a brief summary listing of the numerous toxicological reviews conducted on the diversified toxicity submitted by the registrant to support the safety of his requested tolerances on food and feed:

- acute rat oral LD$_{50}$ (PP# 1F1024) > 10,000 mg/kg
- acute dog oral LD$_{50}$ (PP# 1F1024) > 5,000 mg/kg
- acute rabbit dermal LD$_{50}$ (PP# 1F1024) > 10,000 mg/kg
- rabbit eye irritation (PP# 1F1024) transient irritation
- acute rabbit inhalation LC$_{50}$ (PP# 1F1024) > 4.7 mg/L
- rabbit teratogenic (PP# 9F0743) negative at 62.5 mg/kg (highest fed level)

- 16 Week Dog Feeding NEL < 250 ppm
- 4 Month Rat Feeding (#200-198) NEL < 250 ppm
- 2 Year Dog Feeding NEL < 0.15%
- 2 Year Dog Feeding (#200-206) NEL 60 ppm
- 2 Year Rat Feeding (#200-154) NEL < 0.5%
- 18 Month Rat Feeding (#200-175) NEL < 0.05%
- 2 Year Rat Feeding (#200-205) NEL 60 ppm
- 3 Generation Rat Reproduction (#200-155) NEL < 0.5%
- 3 Generation Rat Reproduction (#200-150) NEL 15,000 ppm (reproduction)

Metabolite Data (DAC-3701-14-hydroxy-2,5,6-trichloroisopthalonitrile)

- Acute Rat Oral LD$_{50}$ (S-D Rats) male 422 mg/kg
- Acute Dog Oral LD$_{50}$ PP # 2F1230 (293-021) 100 mg/kg
- Acute Rat Oral LD$_{50}$ PP # 2F1230 (293-004) 332 mg/kg
- 4 month Rat Feeding PP # 2F1230 (#24-051) NEL 100 ppm
- 90 Day Dog Feeding PP # 2F1230 (#24-052) NEL < 50 ppm
- 3 Generation Rat Reproduction PP # 2F1230 NEL not established

Present Action

The Diamond Shamrock Chemical Company requested in their May 25, 1976 letter from Mr. J. R. Lucietta to our Director PRO that a tolerance of 0.2 ppm be established in soybeans. The additional toxicity data submitted in support of this tolerance were requested by EPA on the metabolite DAC-3701 (letter from Mr. Hamilton H. Parran - EPA to Dr. Milton Eisler of Diamond Shamrock Corp. dated 12/6/72). EPA requested these toxicity data on the metabolite because it appeared in milk when forage treated with chlorothalonil was fed to cows.
Host-Mediated Assay - (DAC 3701) - Brown University - 1/2/74

The material tested was identified as DAC-3701 (4-hydroxy 2,5,6-
trichloroisophthalonitrile with a reference number of 8015-56-14
and a purity of 99.4%. The investigating laboratories were Brown
University and the Genetics Department of Roger Williams General
Hospital. The investigator was Marvin S. Legator.

The test material was diluted to yield a stock solution of 1 mg/ml in
10% DMSO and 90% distilled water. This stock solution was administered
orally to ten mice for five days at the level of 6.5 mg/kg/day. The
bacteria - eight histidine auxotrophs of Salmonella typhimurium -
which were grown to a density of approximately 3.5 x 10^9 cells/ml
(log phase) were injected intraperitoneally at the level of 2.0 ml/
mice three hours prior to sacrifice of the test mice. At sacrifice
the peritoneal cavity was injected with 1-2 ml of sterile saline and
then opened for the removal of the exudate. This exudate was then
diluted, plated and scored.

Results - no increase in number of mutants over the control were
evident at any concentration. The positive control compound, dimethyl
nitrosamine, increased mutation frequency for the 6-46 tester strain
from a single I.P. administration of 4.0 mg/kg.

In Vivo Cytogenetic In Mice - (DAC 3701) - 1/2/74

The material tested was identified as DAC-3701 (4-hydroxy 2,5,6-
trichloroisophthalonitrile with a reference number of 8015-56-14 and
a purity of 99.4%. The investigating laboratories were Brown University
and the Genetics Department of Roger Williams General Hospital. The
investigator Marvin S. Legator used the micronuclei procedure.

The test material was diluted to yield a stock solution of 1 mg/ml in
10% DMSO and 90% distilled water. This stock solution was administered
orally at 6.5 mg/kg/day to ten animals. Three to four hours after the
final administration of the test material, the animals are sacrificed
and air dried slides of the polychromatic cells from the bone marrow
are made. The polychromatic cells (2,000 per treatment) were examined
under 400X magnification for presence of micronuclei.

Results - The controls exhibited 0.38% cells with micronuclei whereas
the DAC 3701 and positive controls (TEM 0.5 mg/kg) animals showed 0.33
and 0.98% cells with micronuclei.

No mutagenic activity is indicated at this level.
**Mice Dominant Lethal Test - (DAC 3701) - 1/2/74**

The material tested was identified as DAC-3701 (4-hydroxy 2,5,6-trichloroisophthalonitrile with a reference number of 8015-56-14 and a purity of 99+%). The investigating laboratories were Brown University and the Genetics Dept. of Roger Williams General Hospital. The investigator was Marvin S. Legator.

This compound was administered to 10 male mice by gavage daily for five days at 6.5 mg/kg/day. Each of these male mice were then mated with two different females each week for eight weeks. The females were sacrificed 12 days from the calculated time of conception.

Observations and tests for effects included fertility, total number of implantations, total number of corpora lutea, preimplantation loss, dead implantation and dead implants/total implants.

Results - The only adverse effect noted was a significant increase in early deaths at week 3 of mating which corresponds to the postmeiotic stage of spermatogenesis (spermatid stage).

**Mice Dominant Lethal - (DAC 3701) 1/17/75**

The material tested was identified as DAC 3701 (4-hydroxy 2,5,6-trichloroisophthalonitrile with a reference number of 8015-56-14 with a purity of 99+%).

This compound was reportedly administered to male mice using the standard procedure for dominant lethal testing of Dr. Marvin Legator. However, the toxicity resulting from the study does not support the alleged protocol.

Results - Since the study does not reveal the total number of male mice utilized at each dosage level, number of weeks the males were mated, number of females mated per male per week, or the conception times, little value is attached to it until the proper data are available.

**Rat Dominant Lethal - DAC 3701 - 8/6/75**

The material tested was identified as DAC-3701 (4-hydroxy 2,5,6-trichloroisophthalonitrile with a purity of 99+%). The reference number is 8015-56-14 and the laboratory project number is 24-101. Dosing suspensions were prepared of DAC 3701 in a 0.5% distilled water solution of hydroxypropycellulose.

The following dosage schedule was followed:
<table>
<thead>
<tr>
<th>Group No.</th>
<th>No. Males</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>2 mg/kg DAC-3701 single dose orally (0.67 ml/kg)</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>4 mg/kg DAC-3701 single dose orally (1.33 ml/kg)</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>8 mg/kg DAC-3701 single dose orally (2.67 ml/kg)</td>
</tr>
<tr>
<td>4 (negative control)</td>
<td>10</td>
<td>Vehicle at 2.67 ml/kg single dose orally.</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>2 mg/kg DAC-3701 5 daily oral doses (0.67 ml/kg)</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>5 mg/kg DAC-3701 5 daily oral doses (1.33 ml/kg)</td>
</tr>
<tr>
<td>7</td>
<td>10</td>
<td>8 mg/kg DAC-3701 5 daily oral doses (2.67 ml/kg)</td>
</tr>
<tr>
<td>8 (negative control)</td>
<td>10</td>
<td>Vehicle at 2.67 ml/kg daily 5 oral doses</td>
</tr>
<tr>
<td>9 (positive control)</td>
<td>10</td>
<td>Cyclophosphamide 40 mg/kg I.P. - one injection</td>
</tr>
</tbody>
</table>

In the acute study, the treated males were mated to two virgin females each week for eight weeks. In the sub-acute study, the treated males were mated to two virgin females each week for seven weeks. The females were sacrificed 12 days after the last day of mating.

Observations and tests for effects included examination of implantation sites, resorption site count, fertility, fetal deaths, implantation site count and live fetuses.

Results - No dominant lethal mutagenic effects were reported.
Rabbit Teratogenic Pilot Study - DAC 3701 - 2/25/76

The material tested was identified as DAC-3701 (4-hydroxy 2,5,6-trichloroisophthalonitrile) with a 99+% purity. The reference number is 8180-70 and the laboratory number is 293-032.

Five pregnant Dutch Belted rabbits were tested per level of 1, 5, 10, 25, and 50 mg/kg/day by oral intubation on days 6 through 18 of gestation. Caesarean sections were performed on the 28th day of gestation.

Observations and tests for effects included general behavior, appearance survival, live fetuses, dead fetuses, empty implantation sites, early resorptions, late resorptions, corpora lutea count, fetal weight and skeletal examination.

Results - No teratogenic effects were evident at the levels tested. Effects per level are as follows:

1 mg/kg/day - no adverse effects
5 mg/kg/day - one female aborted and one female produced a litter of runts
10 mg/kg/day - two females died
25 mg/kg/day - all females died
50 mg/kg/day - all females died

Teratology in Rabbits - (DAC 3701) - 2/25/76

The material tested was identified as DTX-75-0016 by the testing laboratory International Research and Development Corp. The laboratory report number is 293-J32a.

This material was administered by oral intubation to ten pregnant Dutch Belted rabbits per level of 1, 2.5, and 5.0 mg/kg/day on days 6 through 18 of gestation. Caesarean sections were performed on the 28th day of gestation.

Observations and tests for effects included maternal body weights, behavior, implantation site count, fetal deaths, fetal weight, fetal survival, fetal sex ratio, resorptions, corpora lutea count, fetal external anomalies, fetal visceral anomalies and skeletal anomalies.

Results: No significant adverse effects were evident at the 1.0 mg/kg/day level. A dose dependent maternal toxicity was evident at the 2.5 and 5 mg/kg/day in the form of one death and one abort at 2.5 mg/kg/day and 2 deaths and four aborts at 5 mg/kg/day.

No teratogenic effects were reported at tested levels.
The material tested was identified as DAC 3701 (4-hydroxy 2,5,6-trichloroisophthalonitrile) with a purity of 99%. Batch number is 8180-70. Study number is 24-073.

The rats used for this 73-week feeding study were derived from Sprague-Dawley stock of the Spartan strain and were the first offspring (F1a) in a three generation reproduction study using the same dosage levels as the feeding study.

### Animal Groups and Dietary Levels

<table>
<thead>
<tr>
<th>Group No.</th>
<th>No. of Animals</th>
<th>Dietary Levels (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Control)</td>
<td>30 Male 30 Female</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>30 Male 30 Female</td>
<td>10</td>
</tr>
<tr>
<td>III</td>
<td>30 Male 30 Female</td>
<td>50</td>
</tr>
<tr>
<td>IV*</td>
<td>30 Male 26 Female</td>
<td>100</td>
</tr>
<tr>
<td>V*</td>
<td>27 Male 17 Female</td>
<td>200</td>
</tr>
</tbody>
</table>

*Lowered numbers are due to lowered survival at this treatment level in the reproduction study (Bio/Tox 24-051A).

Observations and tests for effects during the study included body weights, food consumption, mortality, tissue mass incidence, urinalysis and the following clinical studies:

- Hematocrit
- Hemoglobin
- RBC
- Total leukocyte count
- Differential leukocyte count
- Sodium
- Potassium
- Chloride
- S. fasting glucose
- S. urea nitrogen
- S. bilirubin
- SAP
- SGOT
- SGPT

Terminal studies included organ weights of the thyroids, liver, kidneys, adrenals, testes with epididymis, heart and spleen and a microscopic examination of the following tissues:
pituitary      large intestine
thyroids       mesenteric lymph
lung           urinary bladder
heart          testes
liver          ovaries
spleen         sternum
kidney         unusual lesions
adrenals       small intestine
stomach        pancreas

Results: The average rat age at termination was 84.7 weeks. During the weeks from 44 to 48, corneal ulcers were noted among all groups and sexes.

This condition declined to virtually zero at week 57 and was followed by corneal opacity in a similar pattern. An infectious or environmental factor is suspected rather than the test material.

The other parameters investigated produced a picture of acceptable biological variations.

The no effect level is 200 ppm.

Note #: Since the dates given in the report did not support the 73 week classification of this study, I called Dr. Budny of Diamond Shamrock Chemical Company and asked for clarification of the timetable. On 12/2/76, Dr. Budny informed me by letter of the timetable. According to his dates the rats appear to have been exposed to the chemical for from 85 to 87 weeks. This does not include the in utero exposure period of 21 days.

According to the criteria under which such experimental testing is conducted, this study satisfies the chronic feeding requirement but not the oncogenic requirement.

Three Generation Rat Reproduction - DAC 3701 - 10/9/75

The material tested was identified as DAC 3701 (4-hydroxy 2,5,6-trichloroisophthalonitrile) - Batch No. 8180-70 with a purity of 99+%

Ten 7 week old males and 20 seven week old females were tested per level of 0, 10, 50, 100 or 200 ppm. After 70 days on these levels, the rats - F0 generation - were mated to produce the F1a generation. The F1a generation were divided into two groups, one of which served as parents for the F2a generation, the other served at the same dosage levels on a two year feeding study. The F0 rats were remated because of poor survival among the F1a pups, to produce the F1b generation. The F2a generation were mated to produce the F3a generation which were carried to weaning.
Observations and tests for parents included body weights, mortality, appearance, behavior, food consumption, fertility, litter size, sex distribution at birth, liveborn, stillborn, abortions and histopathological examination of the following tissues from 5 males and 5 females of each group of the F3 generation:

- thyroid
- heart
- stomach
- lung
- liver
- kidneys
- spleen
- adrenals
- urinary bladder
- gonads

Observations and tests for effects on the pups included mortality, litter weights at 1, 4, 7, 14 and 21 days, individual body weights at 21 days, and skeletal examination of stillborn pups.

Results -

F0 generation - the 200 ppm parent animals appeared to be nervous and irritable. The females of this level also showed a decrease in growth rate. The 10, 50 and 100 ppm levels were unremarkable. The viability index and mean pup weight for both litters (F1a and F1b) of the 200 ppm level were significantly lower than the control value. The lower mean pup weight of the 100 ppm level was also judged to be part of the dose dependent effect.

F1 generation - the 200 ppm parent animals exhibited a significantly lower mean body weight throughout the course of the study. They are reported to have rough hair coats, thin hair coats, pale appearance and irritability. The fertility index was also significantly lower than the control value. The F2a pups from the 200 ppm level also showed a marked reduction in viability.

A dose dependent reduction in the mean litter weights was evident for both the 100 ppm and 200 ppm pups. No adverse findings were reported for the 10 ppm and 50 ppm pups.

F2 generation - the 200 ppm parent animals exhibited lower mean body weights throughout the study (3-17 weeks). However, their body weight gains were comparable to the control value and in the case of the males, it was higher. The fertility index was reduced at both the 100 ppm and 200 ppm levels. Viability of the F3a pups was reduced at the levels of 50, 100, and 200 ppm. The mean litter weights were reduced for the 100 ppm and 200 ppm levels. The number of stillborn was higher in the control group and the low level than in the highest level tested.

Note: This reviewer did not finish study due to numerous poor reporting; conflicting data, missing data, etc.
Summary

The considerations necessary to the decision as to whether or not the voluminous toxicity data generated by the Petitioner and evaluated by various staff members of OPP to support tolerances for daconil are complex to say the least. The existing tolerances as listed in this report were judged to be safe based on the subacute and chronic feeding conducted on the parent compound. The type and extent of these studies were governed by past regulations and reviewer's requirements. The toxicity of the major metabolite -4-hydroxy 2,5,6-trichloro-isonitride, does not jeopardize this safety judgement because of the following information and restrictions generated by the Chemistry Branch.

A) Residue data on the RAC's for which a tolerance has been established could not detect the presence of the 4 hydroxy metabolite above the background parameters in the analytical system utilized.

B) The parent compound is metabolized to the 4 hydroxy metabolite in the soil by the micro-organisms and not by the RAC.

C) The source of the 4-hydroxy metabolite on the vine type RAC appears to be due to soil residues on the harvested RAC.

D) A restriction on using the vine type RAC as a feed item eliminates the possibility of residues in meat and milk.

Due to the increased demand by EPA for additional supportive toxicological information concerning whether or not chemicals will react differently upon life time exposure in other species, the 18 month mouse carcinogenic study has been made a data requirement for tolerances and registrations. This study has not been made available for review at this time by the petitioner and thus the requested tolerance in soybeans cannot be granted. This data gap is listed in the Tuesday, 17, 1976 CFR listing of data requirements to support registration of pesticide active ingredients and preliminary schedule of call-ins.

Another consideration pertinent to the establishment of this tolerance is the allowable daily intake. In his letter of 3/1/71, Dr. H. Blumenthal concluded the safe intake of daconil to be 0.03 mg/kg or approximately 1.8 mg/day in the total daily of 1500 gms for a 60 kg human. He based the ADI of 0.03 mg/kg on a NEL of 60 ppm in the rat and 120 ppm in the dog. However, since that time, Dr. E. Long has determined the "questionable" effects at 120 ppm in the dog to be valid effects. Dr. E. Long has thus determined the NEL to be 60 ppm in the dog. When the 60 ppm value is used in the 120 ppm value in the ADI calculations, the ADI changes from 0.03 mg/kg to 0.015 mg/kg. Thus the safe level of daconil in the 1500 gm daily diet of a 60 kg human is 0.9 mg/day.
(60 kg x 0.015 mg/kg). When this value of 0.9 mg/day is compared to the numerical quantity of daconil contributed to the diet by the existing tolerances (1.13 mg), we find that the calculated ADI has been reached. Thus no additional tolerances which will contribute additional residues of daconil to the daily diet can be established.

The additional toxicity data submitted in the petition are limited to the 4-hydroxy metabolite. These data reveal no effect level of 200 ppm in the chronic rat feeding study. This study is judged to not fulfill the rat carcinogenic requirement due to the length of time the rats were exposed to the test material.

Other data reveal the lack of mutagenic activity in several systems and the lack of teratogenic activity.

A review of the 3 generation rat reproduction revealed a poorly written and prepared document. Many phone calls to the petition (Dr. Budny, D216 352-9311) were made in order to obtain both comments and written data not contained in the report submitted for review. The information supplied by Dr. Budny was even more supportive of a poor study. This reviewer stopped his effects after finding nine inconsistencies on page 105.

This reproduction study is considered invalid for regulatory requirements.

The time table for the reported 120-day rat feeding is also somewhat vague. According to the information provided by Dr. Budny, the study may have been a somewhat longer one—say, about 135 days.