MEMORANDUM

Subject: Review of Acute Delayed Neurotoxicity Study with Endosulfan Technical in the Domestic Hen

To: George LaRocca, Product Manager 15 Registration Division (TS-767)
Through: Robert P. Zendzian, Ph.D., Acting Head Review Section IV

and Theodore M. Farber, Ph.D., Chief Toxicology Branch

From: Margaret L. Jones Review Section IV


Action Requested: Review of the Acute Delayed Neurotoxicity Study on Endosulfan Technical which was identified as a "data gap" in the 1982 Endosulfan Registration Standard.

Background: The neurotoxicity study was submitted to the Agency after being required by the 1982 Endosulfan Registration Standard. The Toxicology Data Tables stated that the neurotoxicity data requirement was only partially satisfied. The table stated that "Endosulfan does not relate to a known group of cholinesterase inhibitors, but there are indications of cholinesterase inhibition. Further testing is therefore required..."

1. Endosulfan Registration Standard, March, 1982, Toxicology Data Requirements Table, Footnote 2/, p. 17.
When the Endosulfan Registration Standard was published in March, 1982, the criteria for requesting neurotoxicity studies were less precise than at present. The Pesticide Assessment Guidelines, Subdivision F, were published in October, 1982 and Data Requirements for Pesticide Registration, Final Rule, 40 FR 157,125 were published in October, 1984. These documents clarify the criteria for requiring acute delayed neurotoxicity testing in the hen. Both sources indicate the only appropriate compounds which should be used in this test are organophosphates or metabolites and degradation products thereof or compounds which are structurally related to substances known to cause acute delayed neurotoxicity.

Conclusions: Under these criteria, testing of Endosulfan which is not an organophosphate or structurally related to substances known to cause acute delayed neurotoxicity would not be required. Although results of the subject neurotoxicity study are inconclusive, in the 9/40 animals examined at 42 days after initial and challenge (day 21) dosing, there was no evidence of progressive nerve damage in brain, spinal, or peripheral nerve. Based on these results and the structure of the compound, no further neurotoxicity testing is required at this time. No conclusions can be made from the results of this study since all animals dying during the study were not examined and several of the suggested Guidelines for conduct of a Neurotoxicity study (Pesticide Assessment Guidelines, 1982, Subpart F, §163.81-7) were not fulfilled.

Core Classification: Supplementary

Recommendations: No further testing in the hen is required.

1. Further discussion of why this study is inconclusive appears in the attached Data Evaluation Report.
DATA EVALUATION REPORT

Chemical: Endosulfan; Thiodan ® Technical

Test Material: Technical grade Endosulfan HOE 002671 01
ZD 97 0003, (97.2% pure).

Study Identification: Acute Delayed Neurotoxicity Study With
Endosulfan - Technical in the Domestic Hen; Roberts, N.L.,
Gopinath, C., Phillips, C.; Unpublished Study conducted by
Huntingdon Research Centre plc; Huntingdon, Cambridgeshire,
England for Hoechst Aktiengesellschaft, Pharma Forschung
Toxicologie, Frankfurt, West Germany; 22 December 1983;
EPA Accession No. 256114, (HST 225, A 28065).

Reviewed By: Margaret L. Jones 
Review Section IV

Approved By: Robert P. Zendzian, Ph.D., Acting Head
Review Section IV

Conclusions: Neurotoxicity assessment with results from this
study is inconclusive since only the animals surviving to day
42 were subjected to histopathology examination. Subdivision F
of the 1982 Pesticide Assessment Guidelines states "All animals
should be subjected to microscopic examination"[§81-7(5)(5)(ii)].
Instead, only 9 survivors of 40 dosed animals were subjected to
histopathology examination. The study is therefore classified
as Supplementary.

Quality Assurance: Q.A. Audit Statement No. HST 225/83888 was
signed by K.W.G. Shillam on 12/20/83.

Materials:

Test substances: Endosulfan - technical, 97.2% pure (HOE
002671 01 ZD 97 0003), Certificate of Analysis No. 02184, 2/1/83.
Tri-ortho-cresyl-phosphate (TOCP); Coalite
Group Limited, Ref. no. S16848.

Test animals: 200 adult domestic hens (Gallus gallus
domesticus) from Graygable Poultry Service, Bury St. Edmunds,
Suffolk; 12 months old at the time of arrival. Body weight
ranged from 2040 g. to 2565 g. at the start of the pre-treatment
period.

Methods: The study began with a preliminary range finding test
followed by an LD50 determination, protection assessment, and
then neurotoxicity assessment. Each phase was preceded by a 14
day observation period.
LD50 determination was performed using 6 groups of 5 hens given 0, 40, 60, 90, 110, and 135 mg/kg by oral gavage.

Protection assessment was performed using 5 groups of 5 hens as follows: 1- Endosulfan 96 mg/kg, 2- Endosulfan 96 mg/kg + phenobarbitone 15 mg/kg, 3- Endosulfan 96 mg/kg + diazepam 2 mg/kg, 4-5- Endosulfan 96 mg/kg + atropine 10 mg/kg + 2 PAM 25 mg/kg.

Neurotoxicity assessment was performed using groups of 10 hens. The vehicle control group was dosed with corn oil, the positive control group was dosed with 500 mg/kg TOCP (12.5% wt/vol), and 4 test groups were dosed with 95 mg/kg Endosulfan (2.4% wt/vol). Dosing was followed by a 21 day observation period, a redosing, and a final 21 day observation period. Surviving hens were sacrificed at 42 days.

Observations were made daily for health, mortality, and ataxia and twice weekly for body weight changes and food consumption. Post mortem examinations were performed on all survivors at 42 days. The tissues examined for evidence of neurotoxicity were stored in 10% buffered formalin. Samples of the following tissues were examined:

Brain- Medulla/pons, cerebellar cortex and cerebral cortex, basal ganglia, hippocampus, other cranial nerves
Spinal cord- Multiple longitudinal and cross sections of the cervical, thoracic, and lumbar-sacral regions
Peripheral nerve- proximal and distal sciatic nerve and tibial nerve (distal branches); 2 cm of proximal axon

The samples were processed with different grades of industrial methylated spirit (64 o.p.), tissue clearing fluid, and paraffin wax (56°). Sections of 8 um were stained with hematoxylin and eosin.

Results:

The LD50 was calculated from the mortalities in Table I using probit analysis. The resulting LD50 was 96 mg/kg (95% Confidence Interval = 69 mg/kg-151 mg/kg).

Table I
Mortalities Following Oral Gavage Dosing for LD50 Determination

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment/(mg/kg)</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Corn Oil 0</td>
<td>0/5</td>
</tr>
<tr>
<td>2</td>
<td>Endosulfan 40</td>
<td>0/5</td>
</tr>
<tr>
<td>3</td>
<td>Endosulfan 60</td>
<td>1/5</td>
</tr>
<tr>
<td>4</td>
<td>Endosulfan 90</td>
<td>2/5</td>
</tr>
<tr>
<td>5</td>
<td>Endosulfan 110</td>
<td>3/5</td>
</tr>
<tr>
<td>6</td>
<td>Endosulfan 135</td>
<td>4/5*</td>
</tr>
</tbody>
</table>

*All four deaths in Group 5 occurred within 2 hours of dosing.
Observed toxic signs were subdued behavior following dosing in all groups which continued in groups 3, 4, 5, and 6 through day 4, and weight loss during the first 7 days of the test period at 110 mg/kg and 135 mg/kg. All groups showed gains in body weight between days 7 - 14.

Results of Protection Assessment are found in Table II.

Table II

Mortalities Following Administration of the LD$_{50}$ Dose Level with Protective Compounds

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment/(mg/kg)</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Endosulfan/96</td>
<td>3/5</td>
</tr>
<tr>
<td>2</td>
<td>Endosulfan/96 + Phenobarbitone/15</td>
<td>2/5</td>
</tr>
<tr>
<td>3</td>
<td>Endosulfan/96 + Diazepam/2</td>
<td>2/5</td>
</tr>
<tr>
<td>4</td>
<td>Endosulfan/96 + Atropine/10 + 2 PAM/25</td>
<td>0/5</td>
</tr>
<tr>
<td>5</td>
<td>Endosulfan/96 + Atropine/10 + 2 PAM/25</td>
<td>2/5</td>
</tr>
</tbody>
</table>

No notable signs of intoxication appeared prior to the 3 deaths in Group 1. In Group 2, all birds became subdued following dosing. One bird died after about 1 hour following a period of violent wing flapping and the second died overnight after remaining subdued. In Group 3 the birds also became subdued and deaths occurred overnight following days 1 and 2. One bird in Group 4 was unsteady following dosing. No toxic signs were apparent in Group 5 immediately following dosing but one hen died overnight and at the end of day 2 one bird was observed sitting on the cage floor, "legs spread out and making violent swaying movements of the head" and subsequently died overnight. Protection was not conclusively demonstrated.

Table III

Mortalities Following Treatment With Endosulfan for Neurotoxicity Assessment

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment/mg/kg</th>
<th>Days 1-21$^2$</th>
<th>Mortality Days 21-42$^3$</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Corn Oil</td>
<td>0</td>
<td>0</td>
<td>0/10</td>
</tr>
<tr>
<td>2</td>
<td>TOC/500</td>
<td>0</td>
<td>0</td>
<td>0/10</td>
</tr>
<tr>
<td>3</td>
<td>Endosulfan/96</td>
<td>3</td>
<td>3</td>
<td>6/10</td>
</tr>
<tr>
<td>4</td>
<td>Endosulfan/96</td>
<td>6</td>
<td>3</td>
<td>9/10</td>
</tr>
<tr>
<td>5</td>
<td>Endosulfan/96</td>
<td>7</td>
<td>1</td>
<td>8/10</td>
</tr>
<tr>
<td>7$^4$</td>
<td>Endosulfan/96</td>
<td>7</td>
<td>1</td>
<td>8/10</td>
</tr>
</tbody>
</table>

2. Deaths occurred on days 1-13.
3. Deaths occurred within 48 hours of redosing.
4. Group 7 replaced group 6 which was discarded due to "bullying" in the group.
Toxic signs included "lethargy, unsteadiness and loss of balance, trembling, wing flapping and leg kicking."\(^1\) The test report states "no signs of ataxia were recorded in the negative control birds or any of the birds dosed with Endosulfan."\(^2\)

**Body Weight changes and Food Consumption**

Mean body weight loss occurred during the 14 day pre-test observation period. Table IV shows the mean changes in body weight which occurred during the 21 days after the first dosing.

**Table IV**

<table>
<thead>
<tr>
<th>Group</th>
<th>Days of Study</th>
<th>0-3</th>
<th>3-7</th>
<th>7-10</th>
<th>10-14</th>
<th>14-17</th>
<th>17-21</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 corn oil</td>
<td></td>
<td>+126</td>
<td>+5</td>
<td>-7</td>
<td>+42</td>
<td>-16</td>
<td>-12</td>
</tr>
<tr>
<td>2 TCOP/500 mg/kg</td>
<td></td>
<td>+86</td>
<td>+2</td>
<td>+47</td>
<td>-4</td>
<td>-53</td>
<td>-113</td>
</tr>
<tr>
<td>3 Endosulfan/96 mg/kg</td>
<td></td>
<td>-13</td>
<td>+10</td>
<td>-10</td>
<td>-2</td>
<td>+22</td>
<td>+58</td>
</tr>
<tr>
<td>4 Endosulfan/96 mg/kg</td>
<td></td>
<td>-10</td>
<td>+27</td>
<td>+68</td>
<td>-2</td>
<td>+45</td>
<td>-29</td>
</tr>
<tr>
<td>5 Endosulfan/96 mg/kg</td>
<td></td>
<td>-103</td>
<td>+42</td>
<td>+26</td>
<td>+29</td>
<td>+5</td>
<td>+72</td>
</tr>
<tr>
<td>7 Endosulfan/96 mg/kg</td>
<td></td>
<td>+30</td>
<td>+14</td>
<td>+36</td>
<td>-106</td>
<td>+61</td>
<td>-96</td>
</tr>
</tbody>
</table>

Mean body weight changes generally reflected mean food consumption for comparable intervals in the study.

**Detailed results of clinical examination and histopathology.**

Appendix 7 (Attachment 1) lists the Grades assigned to nerve damage. Grade I reflects no abnormality in white matter. Grade II indicates some disruption or fragmentation of occasional axons but rare abnormalities in myelin. In Grades III-V, damage increases in quantity and severity. Study authors consider Grade III-V damage to reflect "significant damage" whereas Grade II damage is "background" level.

Neuropathology Grades were I for the majority of animals treated with 96 mg/kg Endosulfan for cranial nerve, brain, spinal cord and peripheral nerve. 5/9 showed Grade II damage in the cervical caudal region of spinal cord and 3/9 showed Grade II damage in cervical cranial region of spinal cord. This level of damage was similar to the negative control group treated with corn oil. Hens treated with 500 mg/kg TCOP showed a higher incidence of Grade II through IV damage. In this group, 7/10 showed Grade III and IV axonal damage in cervical cranial spinal cord; 5/10 showed Grade III and IV damage to tibial distal peripheral nerves.

Discussion:

1. An apparent contradiction exists between p. 1 of the Summary section under "Protection Assessment" and p. 3 of the Condensed Results section under "Protection Assessment" concerning doses tested. The Summary reflects the doses indicated in Treatment Section on p. 10 and throughout the detailed Results Section on pp. 17-19. The groups and treatments indicated on p. 3 Section (b) are apparently incorrect. Tables 4 and 8 show numerous inaccuracies in computing the mean body weight changes during successive intervals of the study. (If the numbers are correct as they appear, the study authors should explain how they were calculated.) These mistakes should be corrected.

2. The signs of toxicity described on page 20 of the test report do not include whether they occurred after the first or second dosing. This information could help in deciding whether this compound causes neurotoxicity.

3. Histopathology was performed on only 9 of 40 dosed animals. These were the survivors to 42 days. The Guidelines state that all animals should be subjected to post mortem examination. The test report states that all animals which died on test were not examined due to the problem of "... artefact caused by autolysis." This appears to be a problem which could have been anticipated and provided for prior to carrying out the study.

1. Test Report A28065, p. 35.
Grade I
No white matter abnormality detected.

Grade II
Disruption or fragmentation of occasional axons. Myelin abnormalities are rare. In general, on any slide of the spinal cord (two longitudinal and one transverse sections), the numbers of altered/degenerate fibres detected varied from one to approximately four. On a slide of peripheral nerve, one or two degenerate fibres were included in this grade.

Grade III
Disruption, fragmentation and distortion of a few axons, most of which were more intensely argyrophilic than the residual normal axons. Changes in myelin sheaths were minimal and usually consisted of small spheroids. In general, slides of cord with five or more, and slides of peripheral nerve with three or more degenerate fibres were recorded in this grade.

Grade IV
Qualitatively similar changes described for Grade V (see below) but affecting only moderate numbers of axons. Extent of change greater than in Grade III.

Grade V
Disruption, fragmentation and distortion of many axons, some of which are more argyrophilic on silver staining than normal axons. Considerable variation in thickness of affected axons with occasional large axon balls. Distortion and fragmentation of myelin sheaths in affected areas with variable numbers of myelinophages. A mild glial/Schwann cell response was occasionally present in the most severely affected areas. In general the extent/distribution was widespread.

0
No tissue examined.