

**A 01**

**CODING FORMS FOR SRC INDEXING**

|                                |  |                      |             |
|--------------------------------|--|----------------------|-------------|
| <b>Microfiche No.</b>          | OTS0001341   |                      |             |
| <b>New Doc ID</b>              | FYI-OTS-1098-1341  | <b>Old Doc ID</b>    | 84990000002 |
| <b>Date Produced</b>           | 10/07/98   | <b>Date Received</b> | 10/07/98    |
|                                |  | <b>TSCA Section</b>  | FYI         |
| <b>Submitting Organization</b> | FRAGRANCE MATERIAL ASSN US   |                      |             |
| <b>Contractor</b>              | TNO, NETHERLANDS   |                      |             |
| <b>Document Title</b>          | INITIAL SUBMISSION: LETTER FROM THE FRAGRANCE MATERIAL ASSN US<br>USEPA REGARDING PRELIMINARY RANGE-FINDING STUDY OF AHTN<br>TOXICITY TO ZEBRAFISH, WITH ATTACHMENTS AND DATED 10/7/98 |                      |             |
| <b>Chemical Category</b>       | TETRAHYDRONAPHTHALENE  |                      |             |

A 03

FYI-1098-1341  
MR 11094

MARZULLA AND MARZULLA  
ATTORNEYS AT LAW  
1350 Connecticut Avenue, NW  
Suite 410  
Washington, DC 20036  
(202) 822-6760  
FAX (202) 822-6774



FYI-98-001341

October 7, 1998

Mr. Edward Gross  
FYI Coordinator  
Office of Toxic Substances  
U.S. Environmental Protection Agency  
401 M Street, N.W.  
Mail Code 7407  
Washington, D.C. 20460

RECEIVED  
OFFICE OF TOXIC  
SUBSTANCES  
98 OCT -7 PM 3:38

Contains No CBI

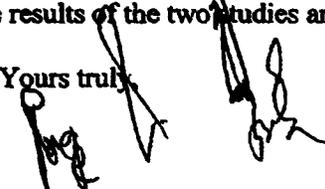
Re: AHTN: Zebrafish Study FYI Filing

Dear Mr Gross:

Enclosed please find a preliminary range-finding study of toxicity of AHTN to zebrafish. My client, the Fragrance Materials Association of the United States, has elected to voluntarily submit this report for inclusion in your database because of the information it contains regarding the unusual occurrence of lesions. You may also find valuable other information regarding the concentration of AHTN used in the study - levels that appear to be significantly above those found in the environment.

In May of 1997, the FMA voluntarily submitted to your office a report of Brixham Laboratories entitled "AHTN: Chronic toxicity to fathead minnow (*Pimephales promelas*) embryos and larvae." The AHTN concentrations in that study were comparable to the levels in this study and the results of the two studies are quite similar.

Yours truly,

  
Roger J. Marzulla

Enclosure



8499000002

A 04

20.97 TUE 16.19 FAX

002

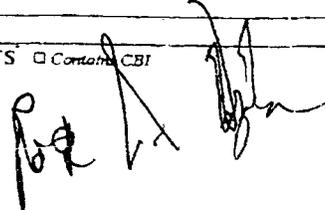
TSCA HEALTH & SAFETY STUDY COVER SHEET - rev. 12-29-96

TSCA CBI STATUS:

CHECK IF THIS PAGE CONTAINS CONFIDENTIAL BUSINESS INFORMATION (CBI)

Clearly mark the confidential information with bracketing and check the box in the appropriate section ( Contains CBI). Submit a sanitized cover sheet with CBI deleted. Mark the sanitized copy, "Public Display Copy" in the heading.

OMB Control # 2070-0156

|   |   |                             |
|---|---|-----------------------------|
| <b>1.0 SUBMISSION TYPE</b> <input type="checkbox"/> Contains CBI<br><input type="checkbox"/> §(d) <input type="checkbox"/> §(e) <input checked="" type="checkbox"/> FYI <input type="checkbox"/> 4 <input type="checkbox"/> OTHER: Specify _____<br><input type="checkbox"/> Initial Submission <input checked="" type="checkbox"/> Follow-up Submission <input type="checkbox"/> Final Report Submission<br>Previous EPA Submission Number or Title if update or follow-up: _____ Docket Number, if any # _____<br><input type="checkbox"/> continuation sheet attached  |   |                             |
| <b>2.1 SUMMARY/ABSTRACT ATTACHED</b><br>(may be required for §(e); optional for §(4), §(d) & FYI)<br><input type="checkbox"/> YES <input checked="" type="checkbox"/> NO  | <b>2.2 SUBMITTER TRACKING NUMBER OR INTERNAL ID</b> | <b>2.3 FOR EPA USE ONLY</b> |
| <b>3.0 CHEMICAL/TEST SUBSTANCE IDENTITY</b> <input type="checkbox"/> Contains CBI<br>CAS# <u>1506-05-1</u> <u>21145-77-7</u> <i>Reported Chemical Name (specify nomenclature if other than CAS name):</i><br>Purity _____ % <i>non-available</i> <u>2-acetyl-1,1,3,4,6-hexamethyl-1,2,3,4</u><br><input type="checkbox"/> Single Ingredient <input type="checkbox"/> Commercial/Tech Grade <input type="checkbox"/> Mixture<br>Trade Name: <u>tetrahydrophthalone</u> Common Name: <u>AHTN</u><br>CAS Number _____ NAME _____ % REIGHT _____<br>Other chemical(s) present in tested mixture: <u>ALONE</u><br><input type="checkbox"/> continuation sheet attached |   |                             |
| <b>4.0 REPORT/STUDY TITLE</b> <input type="checkbox"/> Contains CBI<br><u>Preliminary Fish Early life - stage study with zebrafish</u><br><input type="checkbox"/> continuation sheet attached  |   |                             |
| <b>5.1 STUDY/TSCATS INDEXING TERMS</b><br>[CHECK ONE]<br>HEALTH EFFECTS (HE): _____ ENVIRONMENTAL EFFECTS (EE): <input checked="" type="checkbox"/> ENVIRONMENTAL FATE (EF): _____  |   |                             |
| <b>5.2 STUDY/TSCATS INDEXING TERMS</b> (see instructions for 4 digit codes)<br>STUDY TYPE: _____ SUBJECT ORGANISM (HE, EE only): _____ ROUTE OF EXPOSURE (HE only): _____ VEHICLE OF EXPOSURE (HE only): _____<br>Other: _____ Other: _____ Other: _____  |   |                             |
| <b>6.0 REPORT/STUDY INFORMATION</b> <input type="checkbox"/> Contains CBI <input type="checkbox"/> Study is GLP<br>Laboratory: <u>TNO, The Netherlands</u><br>Report/Study Date: <u>8/25/98</u><br>Source of Data/Study Sponsor (if different than submitter): _____ Number of pages: <u>3</u><br><input type="checkbox"/> continuation sheet attached  |   |                             |
| <b>7.0 SUBMITTER INFORMATION</b> <input type="checkbox"/> Contains CBI<br>Submitter: <u>Roger Marzulla</u> Title: <u>Attorney</u> Phone: <u>(202) 422-6764</u><br>Company Name: <u>Fragrance Materials Assoc.</u> Company Address: <u>1600 J Street, NW</u><br><u>Suite 925, Washington, DC, 20006</u> Submitter Address (if different): <u>1350 Connecticut Ave NW</u><br>Technical Contact: _____ <u>4410, Washington, DC, 20008</u> Phone: ( ) _____<br><input type="checkbox"/> continuation sheet attached   |   |                             |
| <b>8.0 ADDITIONAL/OPTIONAL STUDY COMMENTS</b> <input type="checkbox"/> Contains CBI<br><div style="text-align: center;"> <br/>         Submitter Signature: _____ Date: _____       </div> <input type="checkbox"/> continuation sheet attached   |   |                             |

**MIKE REIVE**  
From: Hooftman@voeding.tno.nl  
Sent: Tuesday, August 25, 1998 3:25 AM  
To: Mike Reive  
Cc: Bowmer@voeding.tno.nl; Borst@voeding.tno.nl  
Subject: RE: Fish ELS test



Dear Mike,

Thank you for your mail.

I can give you the following answers:

- 1 The last five years we did many ELS tests with zebra fish, however we have never observed fish with missing tails before. Curved spines may occur. At the highest concentrations tested some incidental cases of curved spine sometimes occur, but we never saw them so badly curved and in such a high frequency. From experience we know that fish with curved spines mostly does not survive. They are not able to swim normally and probably, due to their disturbed swimming behaviour, they are not capable to catch (enough) food. I suppose they may also suffer sometimes from other disturbances, which cannot be observed visually.
- 2 The foot notes needed indeed a correction. A new version of the document is included. Without applying any statistics I can state the following about the NOEC values:

The NOEC value for condition (including malformations) is most likely 25 microgram per litre  
The NOEC for mortality is most likely 50 microgram per litre.

- 3 The information included in the mail is not an interim report, but just intended to inform you on the status of the preliminary range finding test and the trials with the column and to make agreements for the definitive test. An interim report is not included in this study. According to the protocol only the final test would be reported, and results of the range finding test only when the results are different enough to cause setting of different doses. It will not be a problem to prepare an interim report. However, we will have to consider the GLP status of such a report (excluding data of the column) and the extra costs.
4. In the preliminary range finding test we tested five concentrations (instead of the agreed three concentrations). Exposure to the higher and lower concentration gave us more information about the toxicity, however these concentrations were not analysed. We did only analyse test substance concentrations at  $t = 5$  and  $t = 22$  days for control and validation purposes. More frequent analyses (twice per week) are planned for the definitive final test. The analyses of the range finding test, however, show that the intended actual concentrations achieved are equal to the nominal concentrations.

Van: Mike Reive[SMTp:mike@rlfm.org]  
Verzonden: maandag 24 augustus 1998 23:54  
Aan: Ria Hooftman (E-mail)  
CC: Richard Ford; 'Bowmer@voeding.tno.nl'  
Onderwerp: Fish ELS test

### Results of the (preliminary range finding) toxicity test in the flow through system

Survival of the test animals and their condition  
(the test was started with 40 eggs at each test substance concentration)

| Time | Concentrations in µg/l |        |                |        |      |        |      |                 |      |                 |      |                 |      |                 |
|------|------------------------|--------|----------------|--------|------|--------|------|-----------------|------|-----------------|------|-----------------|------|-----------------|
|      | 0                      |        | 0 <sup>1</sup> |        | 25   |        | 50   |                 | 100  |                 | 200  |                 | 300  |                 |
|      | eggs                   | larvae | eggs           | larvae | eggs | larvae | eggs | larvae          | eggs | larvae          | eggs | larvae          | eggs | larvae          |
| 0    | 40                     |        | 40             |        | 40   |        | 40   |                 | 40   |                 | 40   |                 | 40   |                 |
| 1    | 40                     |        | 40             |        | 40   |        | 40   |                 | 40   |                 | 40   |                 | 40   |                 |
| 2    | 40                     |        | 40             |        | 40   |        | 40   |                 | 40   |                 | 40   |                 | 40   |                 |
| 3    | 18                     | 22     |                | 21     |      | 38     | 4    |                 | 14   |                 | 37   | 3               | 40   | 0               |
| 4    | 0                      | 40     | 1              | 39     | 1    | 39     | 23   | 17              | 18   | 22              | 13   | 27 <sup>4</sup> | 25   | 15 <sup>4</sup> |
| 5    |                        | 40     | 1              | 39     | 0    | 40     | 2    | 38              | 0    | 39              | 0    | 38 <sup>4</sup> | 8    | 32 <sup>4</sup> |
| 6    |                        | 40     | 0              | 40     |      | 40     | 0    | 40              |      | 39              |      | 35 <sup>4</sup> | 0    | 35 <sup>4</sup> |
| 7    |                        | 40     |                | 40     |      | 40     |      | 38              |      | 36              |      | 32 <sup>4</sup> |      | 31 <sup>4</sup> |
| 9    |                        | 40     |                | 40     |      | 40     |      | 38              |      | 33              |      | 27 <sup>4</sup> |      | 1               |
| 12   |                        | 40     |                | 40     |      | 40     |      | 38              |      | 31              |      | 0               |      | 0               |
| 14   |                        | 40     |                | 40     |      | 40     |      | 38              |      | 31              |      |                 |      |                 |
| 16   |                        | 40     |                | 40     |      | 40     |      | 38              |      | 31              |      |                 |      |                 |
| 19   |                        | 40     |                | 40     |      | 40     |      | 38              |      | 31              |      |                 |      |                 |
| 22   |                        | 40     |                | 40     |      | 40     |      | 38              |      | 31              |      |                 |      |                 |
| 25   |                        | 40     |                | 40     |      | 40     |      | 38 <sup>2</sup> |      | 31 <sup>3</sup> |      |                 |      |                 |

- 1 Solvent control (= TEG)
- 2 2 fish were normal; 36 fish had no tail (2 of them were small)
- 3 All fish had no tail
- 4 All fish had a curved spine and were not able to swim normal or to catch food

*Appear to be similar effect ?  
50 µg/l  
no effect until 200*

**Results of the chemical analysis of AHTN in the preliminary range finding test.**

| Time (days)  | Nominal concentration in µg/l | Measured concentration in µg/l |
|--|-------------------------------|--------------------------------|
| <b>In flow through system<sup>1</sup></b>                                |                               |                                |
| 5  | 0                             | 0                              |
| 5  | 50                            | 69                             |
| 5  | 100                           | 103                            |
| 5  | 200                           | 212                            |
| 22   | 50                            | 54                             |
| 22   | 100                           | 105                            |
| <b>column<sup>2</sup></b>  |                               |                                |
| 0  |                               | 991                            |
| 2  |                               | 838                            |
| 4  |                               | 960                            |
| 7  |                               | 941                            |
| <b>solution at effect level prepared by column technique<sup>3</sup></b> |                               |                                |
|  | 69                            | 77                             |

- 1 Samples taken from the flow through system
- 2 Saturated solution prepared by column technique; samples were taken at different running times.
- 3 HPLC pump, provided with column was attached to the mixing vessel of the flow through system

**Conclusions:**

- The findings of Zeneca for fathead minnow are not species specific. Similar effects are observed for zebra fish at the same concentration level.
- Actual concentrations achieved in the flow through system are equal to the nominal values.
- It is possible to produce effective concentrations (without solvent) by column technique.

**CERTIFICATE OF AUTHENTICITY**

**THIS IS TO CERTIFY** that the microimages appearing on this microfiche are accurate and complete reproductions of the records of U.S. Environmental Protection Agency documents as delivered in the regular course of business for microfilming.

Data produced 09 27 1999  
(Month) (Day) (Year)

Mary Furbeck  
Camera Operator

Place Syracuse New York  
(City) (State)

