

Data Evaluation Report on the acute toxicity of Penoxsulam metabolite to aquatic vascular plants *Lemna gibba*
PMRA Submission #: {.....} EPA MRID#: 45831108

Data Requirement: PMRA Data Code: {.....}
EPA DP Barcode: D288160
OECD Data Point: {.....}
EPA MRID: 45831108
EPA Guideline: 122-2

Test material: Penoxsulam Purity: 98%
Common name: 2-amino-8-methoxy XDE-638 metabolite
Chemical name: IUPAC:2-amino-8-methoxy [1,2,4]triazolo[1, 5-C]pyrimidin-5-ol
CAS name: Not reported
CAS No.: Not reported
Synonyms: XDE metabolite 5-OH,2-AMINO-TP

Primary Reviewer: Rebecca Bryan
Staff Scientist, Dynamac Corporation

Signature: *Rebecca Bryan*
Date: 11/21/03

QC Reviewer: Dana Worcester
Staff Scientist, Dynamac Corporation

Signature: *Dana Worcester*
Date: 11/21/03

Primary Reviewer: Bill Erickson
{EPA/OECD/PMRA}

Date: {.....}

J. GOODYEAR *Goodyear*

Secondary Reviewer(s): {.....}
{EPA/OECD/PMRA}

Date: {.....}

Company Code {.....} [For PMRA]

Active Code {.....} [For PMRA]

EPA PC Code 199031 *119031*

Date Evaluation Completed: {dd-mmm-yyyy}

CITATION: Herrera, R. 2002. XDE Metabolite 5-OH,2-AMINO-TP (TSN 101837): Growth Inhibition Test with the Freshwater Aquatic Plant, *Lemna gibba* L. G3. Unpublished study performed by ESG International Inc., Guelph, Ontario, Canada. Laboratory Study No. S2289-01. Study submitted by The Dow Chemical Company, Midland, Michigan, U.S.A. for Dow AgroSciences, LLC, Indianapolis, Indiana, U.S.A. Dow Study No. 021098. Experimental start date April 19, 2002 and experimental termination date May 3, 2002. The final report issued August 2002.



EXECUTIVE SUMMARY:

In a 14-day acute toxicity study, freshwater aquatic vascular plants Duckweed, *Lemna gibba* G3, were exposed to Penoxsulam at a single, nominal concentration of 1.25 mg/L under static conditions. The mean frond numbers, areas under the growth curve, and growth rates were not affected in the 1.25 mg/L treatment group compared to the control. The dry weight percent inhibition was 0.7% for the 1.25 mg/L treatment group. The NOAEC was 1.25 mg a.i./L. The frond number EC₅₀ was >1.25 mg a.i./L. The EC₀₅ could not be determined.

This toxicity study is scientifically sound, but it does not satisfy the U.S. EPA Guideline Subdivision J, §122-2 because the single nominal test concentration, which greatly exceeded the expected environmental concentration (10 µg/L), was not analytically determined. As a result, this study is classified as SUPPLEMENTAL, but it need not be repeated.

Results Synopsis

Test Organism: *Lemna gibba* G3

Test Type: Static

Number of fronds:

NOAEC: 1.25mg/L

EC₀₅: ND

EC₅₀/IC₅₀: >1.25 mg/L 95% C.I.: N/A

Growth rates:

NOAEC: 1.25mg/L

EC₀₅: ND

EC₅₀/IC₅₀: >1.25 mg/L 95% C.I.: N/A

Plant biomass (area under the growth curve):

NOAEC: 1.25mg/L

EC₀₅: ND

EC₅₀/IC₅₀: >1.25 mg/L 95% C.I.: N/A

Dry Weights:

NOAEC: 1.25mg/L

EC₀₅: ND

EC₅₀/IC₅₀: >1.25 mg/L 95% C.I.: N/A

Endpoint(s) Affected: None

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The test was based on the following guidelines: Organization for Economic Cooperation and Development (OECD), Proposal for new Guideline #221, "*Lemna* sp. Growth Inhibition test", OECD Guidelines for Testing of Chemicals; U.S. Environmental Protection Agency, Ecological Effects Test Guidelines, OPPTS 850.4400, Aquatic Plant Toxicity Test Using *Lemna* spp., Tiers I and II EPA 712-C-96-156 (1996); and FIFRA Subdivision J Pesticide Assessment Guidelines, (1982). The following deviations from U.S. EPA Guideline 123-2 are noted:

1. The study was conducted with one test concentration (limit test), which was not analytically determined. As a result, it does not satisfy the Tier I §122-2 guidelines.
2. The pretest health of the test organism was not reported.
3. The dilution water characteristics were not reported.
4. The number of plants (3) was less than recommended, so the number of fronds (12) was less than the recommended 15 fronds per replicate.
5. The stability of the test substance was not determined.

These deviations were not considered to have affected the results of the study, but the failure to analyze the single test concentration affected the acceptability of the study.

COMPLIANCE: Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided.

A. MATERIALS:

1. Test Material Penoxsulam metabolite (5-OH-AMINO-TP)

Description: Beige powder

Lot No./Batch No. : 199900606-27B

Purity: 98%

Stability of Compound

Under Test Conditions: The test concentration was not measured during the study, therefore, stability was not determined. OECD requirements were not reported.

(OECD requires water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound)

Storage conditions of test chemicals: Stored at room temperature.

2. Test organism:

Name: Duckweed, *Lemna gibba* L. (EPA requires a vascular species: *Lemna gibba*.)

Strain, if provided: G3 1913

Source: Laboratory cultures (original supplier: UTCC #310)

Age of inoculum: 10-14 days old (p. 16)

Method of cultivation: 20X AAP Medium

B. STUDY DESIGN:

a) Range-finding Study: No range-finding study was conducted.

b) Definitive Study

Table 1 . Experimental Parameters

Parameter	Details	Remarks Criteria
Acclimation period: culturing media and conditions: (same as test or not) health: (any toxicity observed)	At least 3 weeks 20X AAP Medium; same as test. Not reported	
Test system static/static renewal/ renewal rate for static renewal:	Static	EPA expects the test concentrations to be renewed every 3 to 4 days (one renewal for the 7 day test, 3-4 renewals for the 14 day test).
Incubation facility	Growth chamber	
Duration of the test	14 days	EPA requires a

Parameter	Details	Remarks Criteria
		<i>duration of 14 days. Seven day studies will be accepted for review by the Agency.</i>
Test vessel material: (glass/polystyrene) size: fill volume:	Glass Erlenmeyer flasks 500 mL 200 mL	Test vessels covered with Jaece® non-toxic foam plugs.
Details of growth medium name: pH at test initiation: pH at test termination: Chelator used: Carbon source:	Modified 20X AAP Medium 8.16-8.39 9.43-9.60 Not reported Not reported	<i>EPA recommend the following culture media: Modified hoagland's E+ or 20X-AAP.</i>
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	Not applicable	
Dilution water source/type: pH: water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	20X AAP Medium 7.5 ± 0.1 N/A N/A N/A N/A N/A N/A	The dilution water characteristics were not reported. <i>EPA recommends a pH of ~5.0. A solution pH of 7.5 is acceptable if type 20X-AAP nutrient media is used.</i>
Indicate how the test material is added to the medium (added directly or used stock solution)	Stock solution	
Aeration or agitation	Not reported.	

Parameter	Details	Remarks Criteria
Sediment used (for rooted aquatic vascular plants) origin: textural classification (% sand, silt and clay): organic carbon (%): geographic location:	Not applicable	
Number of replicates control: solvent control: treatments:	6 N/A 6	
Number of plants/replicate	3	EPA requires 5 plants.
Number of fronds/plant	4 fronds per plant (12 total fronds per replicate)	EPA requires 3 fronds per plant.
Test concentrations nominal: measured:	0 (negative control) and 1.25 mg/L Not determined	The measured test concentration was not determined. EPA requires at least 5 test concentrations with a dose range of 2X or 3X progression.
Solvent (type, percentage, if used)	N/A	
Method and interval of analytical verification	N/A	
Test conditions temperature: photoperiod: light intensity and quality:	22.0-26.5°C continuous light 6,500-8,000 lux, cool-white fluorescent light	EPA temperature: 25 °C EPA photoperiod: continuous EPA light: 5.0 Klux (±15%)

Parameter	Details	Remarks Criteria
Reference chemical (if used) name: concentrations:	Zinc Chloride Not reported	The reported EC ₂₅ (95% confidence intervals) was 2.19 mg/L (1.25-3.41 mg/L).
Other parameters, if any	None	

2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks/Criteria
Parameters measured (eg: number of fronds, plant dry weight or other toxicity symptoms)	Number of fronds (number of colonies), dry weights, and toxicity symptoms.	
Measurement technique for frond number and other end points	Direct counts using a hand-magnifying lens.	
Observation intervals	0, 3, 5, 7, 10, and 14 days.	3 replicates harvested on day 7 and 3 replicates harvested on day 14.
Other observations, if any	Area under the growth curve and growth rates.	
Indicate whether there was an exponential growth in the control	Yes	
Were raw data included?	Replicate data provided.	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

The mean frond numbers, areas under the growth curve, and growth rates were not affected in the 1.25 mg/L treatment group compared to the control. The dry weight percent inhibition was 0.7% for the 1.25 mg/L treatment group.

Table 3: Effect of Penoxsulam metabolite on frond number and dry weight of Duckweed, *Lemna gibba*

Treatment nominal concentration mg/L	Initial frond number/ test solution	Mean frond number at				Mean dry weight (mg)	Mean Growth Rate	Mean Area Under the Growth Curve
		3 days	7 days	14 days	% inhibition at 14 days			
Negative	12	30.8	152.3	700.7	---	65.14	0.01214	66,843.67

Acute toxicity of Penoxsulam metabolite to aquatic vascular plants *Lemna gibba*- MRID 45831108

control (dilution water)								
1.25	12	30.8	160.0	720.7	-2.9	64.69	0.01222	73,126.04
Reference chemical (if used)	Not applicable							

Negative percent inhibition represents positive growth.

NR-Not reported

Table 4: Statistical endpoint values.

Statistical Endpoint ^a	frond No.	Dry weight	growth rate	area under the growth curve
NOAEC or EC ₀₅ (mg/L)	1.25	1.25	1.25	1.25
LOAEC (mg/L)	>1.25	>1.25	>1.25	>1.25
EC ₅₀ (mg/L) (95% C.I.)	>1.25	>1.25	>1.25	>1.25
EC ₂₅ (mg/L) (95% C.I.)	>1.25	>1.25	>1.25	>1.25
Reference chemical NOAEC IC ₅₀ /EC ₅₀	Not applicable		Not applicable	Not reported

^a Statistical data based on nominal test concentrations.

B. REPORTED STATISTICS: Percent inhibition was determined for all endpoints. The 14 day NOAEC, and EC₅₀ values were estimated using the significance data for all endpoints. All statistical calculations were performed using the nominal concentration.

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical analyses were not required to verify the results of this study, as there were no reductions in the treatment group for any endpoint.

Number of fronds:

NOAEC: 1.25mg/L

EC₀₅: ND

EC₅₀/IC₅₀: >1.25 mg/L 95% C.I.: N/A

Growth rates:

NOAEC: 1.25mg/L

EC₀₅: ND

EC₅₀/IC₅₀: >1.25 mg/L 95% C.I.: N/A

Plant biomass (area under the growth curve):

NOAEC: 1.25mg/L

EC₀₅: ND
EC₅₀/IC₅₀: >1.25 mg/L 95% C.I.: N/A

Dry Weights:

NOAEC: 1.25mg/L
EC₀₅: ND
EC₅₀/IC₅₀: >1.25 mg/L 95% C.I.: N/A

Endpoint(s) Affected: None

D. STUDY DEFICIENCIES:

While no toxicity was shown in this study and the nominal concentration greatly exceeded the expected environmental concentration (10 µg/L), this test level was not analytically determined. As a result, this study does not satisfy the U.S. EPA Guideline Subdivision J, §122-2 for a limit test with a metabolite.

E. REVIEWER'S COMMENTS:

The reviewer's conclusions agreed with the study author's; there was no toxicity of XDE-638 Metabolite 5-OH, 2-Amino-TP (TSN 101837) to duckweed. While no toxicity was detected in this study and a concentration which greatly exceeded the expected environmental concentration (10 µg/L) was tested, this study cannot be classified as Core because the test concentration was not analytically verified.

The projected environmental concentration (PEC) for 5-OH,2-AMINO-TP is 10 µg/L.

The doubling time of the control was used as validity criteria (OECD). An increase of approximately 8-fold in seven days is required.

The colony number percent inhibition was -6.0% in the 1.25 mg/L treatment group after 14 days (no effect).

F. CONCLUSIONS: This toxicity study is scientifically sound, but it does not satisfy the U.S. EPA Guideline Subdivision J, §122-2 because the single nominal test concentration, which greatly exceeded the expected environmental concentration (10 µg/L), was not analytically determined. As a result, this study is classified as SUPPLEMENTAL, but it need not be repeated

Number of fronds:

NOAEC: 1.25mg/L
EC₀₅: ND
EC₅₀/IC₅₀: >1.25 mg/L 95% C.I.: N/A

Growth rates:

NOAEC: 1.25mg/L

EC₀₅: ND

EC₅₀/IC₅₀: >1.25 mg/L 95% C.I.: N/A

Plant biomass (area under the growth curve):

NOAEC: 1.25mg/L

EC₅₀/IC₅₀: >1.25 mg/L 95% C.I.: N/A

Dry Weights:

NOAEC: 1.25mg/L

EC₀₅: ND

EC₅₀/IC₅₀: >1.25 mg/L 95% C.I.: N/A

Endpoint(s) Affected: None

III. REFERENCES:

- American Society for Testing and Materials (ASTM). 1998. Standard guide for conducting static acute toxicity tests with *Lemna gibba* G3. Annual Book of ASTM Standards. American Society for Testing and Materials. West Conshohocken, PA. Vol. 11.05. E 1415-91 (Reapproved 1998).
- Dow AgroSciences. 2000. Research Sample Safety Data Sheet. Product: XDE-638. Indianapolis, IN. Effective Date: 1/25/00.
- Dow AgroSciences. 2002a. Test Substance Distribution Certificate. Test Substance Number: TSN101837. Indianapolis, IN. Last Assay Date: 03 Jan 2002. Sampled by Kent Hensley 2-26-02.
- Dow AgroSciences. 2002b. Certificate of Analysis for Test/Reference/Control Substances. TSN101837. Indianapolis, IN. Study Completion Date: Jan 3, 2002.
- ESG International Standard Operating Procedures Manual (ESG). 2001a. SOP# 265. Standard Operating Procedures for the Axenic Laboratory Culture of *Lemna gibba* L. G3. ESG International Specialty Chemicals Division Standard Operating Procedures Manual.
- ESG International Standard Operating Procedures Manual (ESG). 2001b. SOP# 345. Standard Operating Procedures for Conducting Growth Inhibition Tests Using *Lemna gibba* L. G3. ESG International Specialty Chemicals Division Standard Operating Procedures Manual.
- ESG International Standard Operating Procedures Manual (ESG). 2001c. SOP# 11. Data Analysis. ESG International Specialty Chemicals Division Standard Operating Procedures Manual.
- ESG International Standard Operating Procedures Manual (ESG). 2001d. SOP# 12. Interpretation and Recording of Reference Toxicant Data. ESG International Specialty Chemicals Division Standard Operating Procedures Manual.
- Federal Insecticide, Fungicide and Rodenticide Act (FIFRA). 1982. Pesticide Assessment Guidelines. Subdivision J. Hazard Evaluation: Nontarget Plants. By R.W. Holst and T.C. Ellwanger. Office of Pesticide Programs. Washington, D.C. 55 pp.
- Organization for Economic Cooperation and Development (OECD). 1998. The OECD Principles of Good Laboratory Practice. Environment Monograph No. 45. Environment Directorate, Paris, France.

Organization for Economic Cooperation and Development (OECD). 2000. Proposal for a new Guideline #221, "*Lemna* sp. Growth Inhibition Test". OECD Guidelines for Testing of Chemicals.

U.S. Environmental Protection Agency (U.S. EPA). 1996. Ecological Effects Test Guidelines. OPPTS 850.4400 Aquatic Plant Toxicity Test Using *Lemna* spp., Tiers I and II. EPA 712-C-96-156.

WEST, Inc., and D.D. Gulley. 1996. TOXSTAT™ 3.5, Computer software and instruction manual. Western EcoSystems Technology, Inc. Cheyenne, Wyoming.