Importance of Triazine Metabolite Determination

Pesticides are frequently applied in agriculture to protect crops from pests and to protect the yield of the harvest. However, a part of the active substance does not reach the target plant but evaporates during application or remains in the soil and degrades into metabolites. Due to their wide application and relatively high persistence, they can be detected in rain, surface water, and ground water. Atrazine and its metabolites are among the more frequently detected compounds in surface and ground water in the United States. It is desirable to monitor water samples and food for residues of triazines. According to the USEPA SWDA drinking water guidelines, the MCL for atrazine in drinking water is 3 ppb. Triazine metabolites are listed on the Drinking Water Contaminant Candidate List 2.

The DACT ELISA allows the determination of 41 samples in duplicate determination. Only a few uL of sample are required. The test can be performed in about 1 hour.

Performance Data

Test sensitivity: The detection limit for DACT is 0.1 ng/mL (90% B/Bo). The concentration of residue necessary to cause a 50% inhibition (50% B/B_0) is approximately1.3 ng/mL. Determinations closer to the middle of the calibration range of the test yields the most accurate results.

Test reproducibility: Coefficients of variation (CVs) for standards: <10%, CVs for samples: <15%.

Selectivity: The ELISA for DACT recognizes DACT and other triazine metabolites.

Cross-reactivities:

DACT	100% (per definition)
Atrazine Despropyl	8.5%
Atrazine Desethyl	2.3%
Cryomazine	2.8%

Atrazine, Cyanazine, Prometon, Propachlor, Propazine, Terbutryne, Atrazine 2-OH, Atrazine Desethyl 2-OH exhibited no reactivity until greater than 1,000 ppb.

Cross-reactivities with pesticide classes other than triazines have not been observed.

Samples: Drinking water, ground water, and surface water were tested for matrix effects in the ELISA. No matrix effects were determined.

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Triazine Metabolite ELISA (Microtiter Plate)

Enzyme-Linked Immunosorbent Assay for the Determination of Diaminochlorotriazine (DACT) in Water Samples

Product No. 520006

1. General Description

The Triazine metabolite ELISA is an immunoassay for the quantitative and sensitive detection of diaminochlorotriazine and other triazine herbicide metabolites. This test is suitable for the quantitative and/or qualitative detection of these metabolites in water samples. A previous sample preparation is not required. For other sample matrices, contact Abraxis for application bulletins and/or specific matrix validation guidelines.

2. Safety Instructions

The standard solutions in the test kit contain diaminochlorotriazine. In addition, the substrate solution contains tetramethylbenzidine and the stop solution contains diluted sulfuric acid. Avoid contact of stopping solution with skin and mucous membranes. If these reagents come in contact with the skin, wash with water. Consult state, local and federal regulations for proper disposal of all reagents.

3. Storage and Stability

The DACT ELISA should be stored in the refrigerator (4–8°C). The solutions must be allowed to reach room temperature (20-25°C) before use. Reagents may be used until the expiration date on the box.

4. Test Principle

The test is based on the recognition of DACT by specific antibodies. DACT present in the sample and a triazine-enzyme-conjugate compete for the binding sites of the antibodies immobilized on the plate. After a washing step and addition of the substrate solution, a color signal is produced. The intensity of the blue color is inversely proportional to the concentration of the DACT present in the sample. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.

5. Limitations of the DACT ELISA, Possible Test Interference

Numerous organic and inorganic compounds commonly found in water samples have been tested and found not to interfere with this test. However, due to the high variability of compounds that might be found in water samples, test interferences caused by matrix effects can't be completely excluded. Mistakes in handling the test also can cause errors. Possible sources for such errors can be:

Inadequate storage conditions of the test kit, wrong pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, extreme temperatures during the test performance (lower than 10°C or higher than 30°C).

The Abraxis DACT ELISA kit provides screening results. As with any analytical technique (GC, HPLC, etc.) positive samples requiring some action should be confirmed by an alternative method.

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Working Instructions

A. Test Preparation

Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary. We recommend using a multi-channel pipette or a stepping pipette for adding the enzyme conjugate, the substrate solution and the stop solution in order to equalize the incubations periods of the standard solutions and the samples on the entire microtiter plate. Please only use the reagents and standards from one package lot in one test, as they have been adjusted in combination.

1. Adjust the microtiter plate and the reagents to room temperature before use.
2. Remove the number of microtiter plate strips required from the foil pouch. The remaining strips are stored in the foil pouch and ziplocked closed. Store the remaining kit in the refrigerator (4-8°C).
3. The standard solutions, positive and negative controls, enzyme conjugate, substrate and stop solution are ready to use and do not require any further dilutions.
4. Dilute the wash buffer at a ratio of 1:5. If using 100 mL of concentrate, add to 400 mL of deionized or distilled water.
5. The stop solution should be handled with care as it contains diluted H_2SO_4.

B. Assay Procedure

1. Add 100 µL of the standard solutions, the control or the samples into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.
2. Add 50 µL of enzyme conjugate solution to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a rapid circular motion on the benchtop for about 30 seconds. Be careful not to spill contents.
3. Incubate the strips for 30 min at room temperature.
4. After incubation, remove the covering and vigorously shake the contents of the wells into a sink.
5. Wash the strips four times using the 1X washing buffer solution. Please use at least a volume of 250 µL of washing buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.
6. Add 100 µL of substrate solution to the wells. The strips are incubated for 30 min at room temperature. Protect the strips from sunlight.
7. Add 100 µL of stop solution to the wells in the same sequence as for the substrate solution.
8. Read the absorbance at 450 nm using a microplate ELISA photometer within 15 minutes after the addition of the stopping solution.

C. Evaluation

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs (4-parameters (preferred) or Logit/Log). For a manual evaluation calculate the mean absorbance value for each of the standards. Calculate the $\%B/B_0$ for each standard by dividing the mean absorbance value of each of the standards by the Zero Standard (Standard 0) mean absorbance. Construct a standard curve by plotting the $\%B/B_0$ for each standard on a vertical linear (y) axis versus the corresponding DACT concentration on horizontal logarithmic (x) axis on graph paper. $\%B/B_0$ for controls and samples will then yield levels in ppb of DACT by interpolation using the standard curve.
The concentrations of the samples are determined using this standard curve. Samples showing a lower concentration of DACT compared to standard 1 (0.125 ng/mL) are considered as negative. Samples showing a higher concentration than standard 5 (5 ng/mL) must be diluted further to obtain accurate results. The concentration of the control should be in the range given on the test vial (±20%).

D. Additional Materials (not delivered with the test kit)

1. Micro-pipettes with disposable plastic tips (50-200 µL, 100-1000 µL)
2. Multi-channel pipette (50-250 µL) or stepper pipette with plastic tips (50-250 µL)
3. Microtiter plate washer (optional)
4. Microtiter plate reader (wavelength 450 nm)
5. Shaker for microtiter plates (optional)

E. Working Scheme

The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test. The standards must be run with each test. Never use the values of standards which have been determined in a test performed previously.

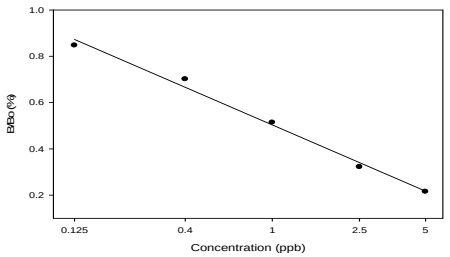
St0-St5: Standards
(0, 0.125, 0.40, 1.0, 2.5, 5.0 ng/mL)

PC (Positive Control): 3 ng/mL +/- 20%

Sam1, Sam2, Sam3, etc.: Samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 0	Std 4	Sam 2		etc.	etc.						
B	Std 0	Std 4	Sam 2									
C	Std 1	Std 5	Sam 3									
D	Std 1	Std 5	Sam 3									
E	Std 2	PC										
F	Std 2	PC										
G	Std 3	Sam 1										
H	Std 3	Sam 1										

F. Standard Curve (These values are used for demonstration purposes; do not use these values for your determinations)



G. References

(1) A. Dankwardt, E.M. Thurman, B. Hock, Terbuthylazine and deethylterbuthylazine in rain and surface water – Determination by enzyme immunoassay and gas chromatography/mass spectrometry, Acta hydrochim. hydrobiol. 25, 1997, 5-10.

(2) A. Dankwardt, S. Pullen, S. Rauchalles, K. Kramer, F. Just, B. Hock, Atrazine residues in soil two years after the atrazine ban – A comparison of enzyme immunoassay with HPLC, Anal. Lett. 28, 1995, 621-634.

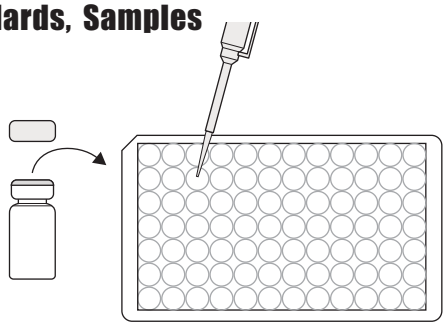
(3) S. Wüst, B. Hock, A sensitive enzyme immunoassay for the detection of atrazine based upon sheep antibodies, Anal. Lett. 25, 1992, 1025-1037.

(4) B. Hock, T. Giersch, A. Dankwardt, K. Kramer, S. Pullen, Toxicity Assessment and On-line monitoring: Immunoassays, Environ. Toxicol. Water Qual. 9, 1994, 243-262.

Triazine Metabolite Plate, Detailed ELISA Procedure

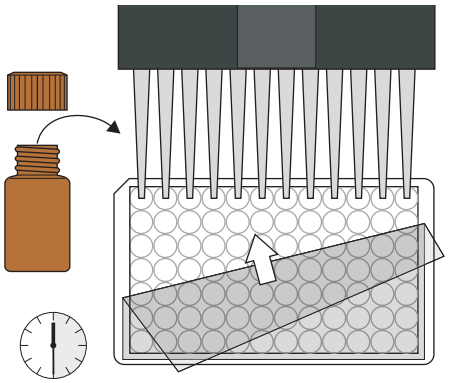
1. Addition of Standards, Samples

Add 100 uL of the standard solutions or samples into the wells of the test strips according to the working scheme given. Be sure to use a clean pipet tip for each.



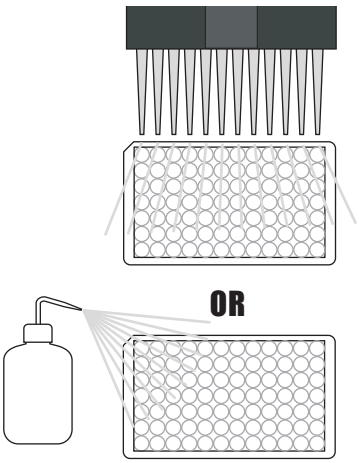
2. Addition of Enzyme Conjugate

Add 50 uL of the Enzyme Conjugate to the individual wells successively using a multi- channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a rapid circular motion on the benchtop for 30 seconds. Be careful not to spill contents. Incubate the strips for 30 min at room temperature.



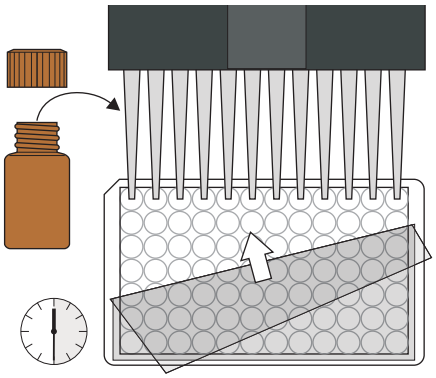
3. Washing of Plates

After incubation, remove the covering and vigorously shake the contents of the wells into a sink. Wash the strips four times with a multi-channel pipette or wash bottle using the diluted 5X washing buffer solution. Please use at least a volume of 250 uL of washing buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.



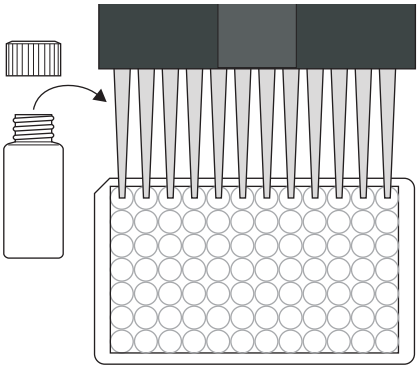
4. Addition of Substrate/Color Solution

Add 100 uL of substrate/color solution to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a rapid circular motion on the benchtop. Be careful not to spill contents. Incubate the strips for 30 min at room temperature.



5. Addition of Stopping Solution

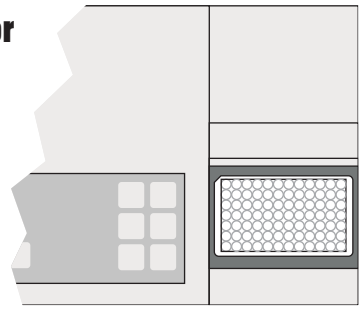
Add 100 uL of stop solution to the wells in the same sequence as for the substrate solution using a multi- channel pipette or a stepping pipette.



6. Measurement of Color

Read the absorbance at 450 nm using a microplate ELISA reader. Calculate results.

NOTE: Multiply ELISA results by appropriate dilution factors.



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Triazine Metabolite Plate, Concise ELISA Procedure

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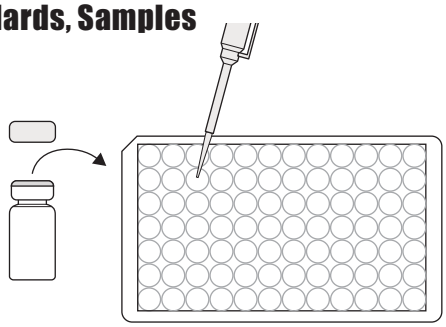
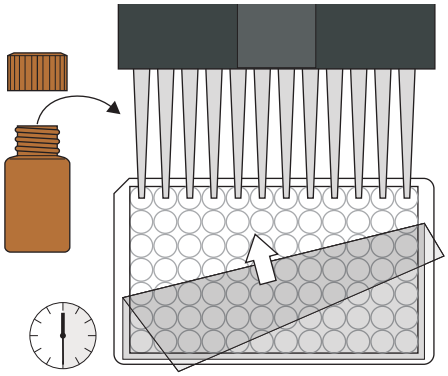
1. Addition of Standards, Samples

Add 100 uL of standard solutions or samples.



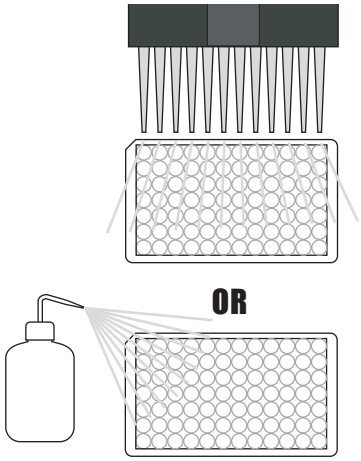
2. Addition of Enzyme Conjugate

Add 50 uL of the Enzyme Conjugate. Cover and mix for 30 seconds by rotating on benchtop. Incubate for 30 minutes at room temperature.



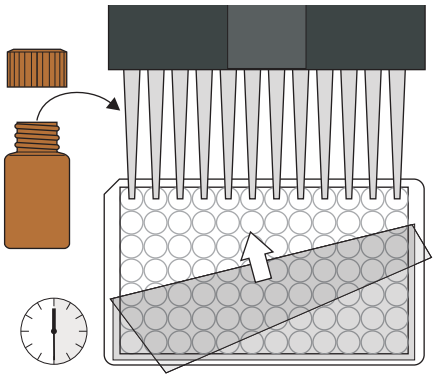
3. Washing of Plates

Wash the plates four times with 250 uL of diluted 1X washing buffer.



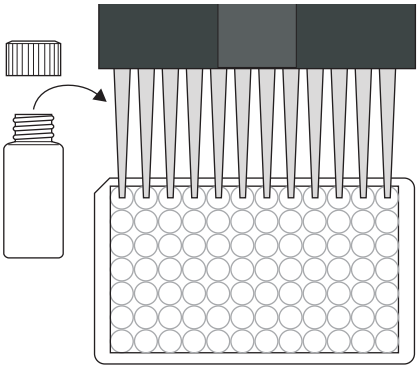
4. Addition of Substrate/Color Solution

Add 100 uL of substrate/color solution. Incubate 30 minutes at room temperature and away from direct sunlight.



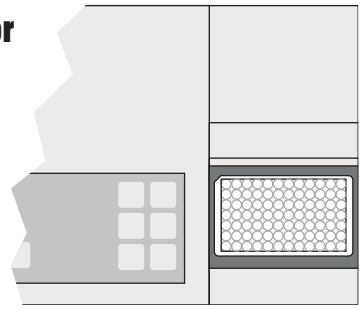
5. Addition of Stopping Solution

Add 100 uL of stop solution.



6. Measurement of Color

Measure color at 450 nm.
Calculate results.



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Safety Data Sheet

Section 1: Product and Company Identification

1.1 Product Identifiers:
Product Name: Triazine Metabolite (DACT) ELISA Plate Kit
Product Code: 520006
1.2 Identified Use: Determination of Triazine Metabolite (DACT) in samples. **Restrictions on Use:** For research use only.
1.3 Company: Abraxis, Inc., 124 Railroad Drive, Warminster, PA 18974 USA, info@abraxiskits.com +1(215) 357-3911, FAX +1(215) 357-5232
1.4 Emergency Telephone Number: +1(215) 357-3911

Section 2: Hazard(s) Identification

2.1 Classification of the mixture: Not a hazardous mixture.
2.2 GHS Label elements, including precautionary statements: Not applicable.
2.3 Hazards not otherwise classified (HNOC) or not covered by GHS: None known.
2.4 Unknown acute toxicity: None known.

Section 3: Composition / Information on Ingredients

3.2 Mixtures: *Contains no hazardous ingredients at levels requiring disclosure by the OSHA Hazard Communication Standard (29 CFR 1910.1200)*, however it contains minor amounts of materials considered hazardous. We recommend handling all substances with caution.

Section 4: First Aid Measures

4.1 Description of first aid measures: Consult a physician. Show this safety data sheet to the doctor in attendance. Move out of dangerous area.
If inhaled: If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.
In case of skin contact: Wash off with soap and plenty of water. Consult a physician.
In case of eye contact: Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.
If swallowed: Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.
4.2 Most important symptoms and effects, both acute and delayed: No data available
4.3 Indication of any immediate medical attention and special treatment needed: No data available. Treat symptomatically.

Section 5: Fire-fighting Measures

5.1 Suitable extinguishing media: Use an extinguishing agent suitable for the surrounding fire.
5.2 Special hazards arising from the substance or mixture: None known
5.3 Advice for firefighters: Wear self-contained breathing apparatus for fire-fighting if necessary.
5.4 Further information: No data available

Section 6: Accidental Release Measures

6.1 Personal precautions, protective equipment and emergency procedures: Use personal protective equipment (see section 8). Avoid dust formation. Avoid breathing vapors, mist, dust, or gas. Ensure adequate ventilation. Evacuate personnel to safe areas.
6.2 Environmental precautions: Prevent further leakage or spillage if safe to do so. Do not let product enter drains. Discharge into the environment must be avoided.
6.3 Methods and materials for containment and cleaning up: Solids (if applicable): Pick up and arrange disposal without creating dust. Sweep up and shovel. Liquids (if applicable): Absorb with liquid-binding material (sand, diatomite, acid binders, universal binders, sawdust). Keep in suitable, closed containers for disposal.
6.4 Reference to other sections: For information on safe handling see section 7.
For information on personal protection see section 8.
For information on disposal see section 13.

Section 7: Handling and Storage

7.1 Precautions for safe handling: See section 2. Avoid inhalation of vapors and contact with skin and eyes. Wear appropriate personal protective equipment. Do not eat, drink, or smoke in work area.
7.2 Precautions for safe storage: Keep container(s) tightly closed in a dry, well-ventilated place. Protect from physical damage. See label or product insert for appropriate storage temperature and additional specific information.

7.3 Specific end use(s): No data available

Section 8: Exposure Controls / Personal Protection

8.1 Control parameters: Not applicable.
8.2 Exposure controls:
Appropriate engineering controls: Provide adequate ventilation. Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of workday. Keep away from food and beverages.
Personal protective equipment: The usual precautionary measures, including eye/face/skin protection, should be taken when handling any chemical. Avoid contact with eyes, skin, and clothing.
Eye protection: As with handling of any chemical, wear approved safety goggles.
Skin protection: Handle with gloves. No specific information regarding glove material or thickness is available, but gloves must be impermeable and resistant to the substance being handled. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.
Respiratory protection: As with any chemical, where excessive vapor, mist, or dust may result, use a chemical fume hood or approved respiratory protection equipment.
Body protection: Lightweight, protective clothing.

Section 9: Physical and Chemical Properties

9.1 Information on basic physical and chemical properties
Appearance: Multiple
Odor: Characteristic
Odor Threshold: No data available
pH: Multiple
Melting point/freezing point: No data available
Initial boiling point and boiling range: No data available
Flash point: Not applicable
Evaporation rate: No data available
Flammability (solid, gas): No data available
Upper/lower flammability or explosive limits No data available
Vapor pressure: No data available
Vapor density: No data available
Relative density: No data available
Water solubility: Various
Partition coefficient: n-octanol/water: No data available
Auto-ignition temperature: Not applicable
Decomposition temperature: No data available
Viscosity: No data available
Explosive properties: No data available
Oxidizing properties: No data available
9.2 Other information: No data available

Section 10: Stability and Reactivity

10.1 Reactivity: No data available
10.2 Chemical stability: Stable under recommended storage conditions.
10.3 Possibility of hazardous reactions: No data available
10.4 Conditions to avoid: No data available
10.5 Incompatible materials: No data available
10.6 Hazardous decomposition products: No data available. In the event of fire: see section 5.

Section 11: Toxicological Information

11.1 Information on toxicological effects
Acute toxicity: Not available. To the best of our knowledge, the chemical, physical, and toxicological properties of this product have not been thoroughly investigated.
Inhalation: No data available **Ingestion:** No data available **Skin contact:** Irritant to skin and mucous membranes.
Eye contact: May cause eye irritation in susceptible persons. **Respiratory or skin sensitization:** No data available
Aspiration hazard: No data available **Mutagenicity:** No data available

Carcinogenicity
IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.
ACGIH: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH.
NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.
OSHA: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.
Teratogenicity: No data available
Reproductive/fertility toxicity: No data available
Specific target organ toxicity, single exposure: No data available
Specific target organ toxicity, repeated exposure: No data available

Section 12: Ecological Information

12.1 Toxicity: No data available
12.2 Persistence and degradability: No data available
12.3 Bioaccumulative potential: No data available
12.4 Mobility in soil: No data available
12.5 Results of PBT and vPvB assessment: No data available
12.6 Other adverse effects: An environmental hazard cannot be excluded in the event of unprofessional handling or disposal.

Section 13: Disposal Considerations

13.1 Waste treatment methods
Product: All waste must be handled and disposed according to local, state, and federal regulations. Avoid disposing large volumes in sewer.
Contaminated packaging: All waste must be handled and disposed according to local, state, and federal regulations.
Refer to sections 7 and 8 for safe handling guidance.

Section 14: Transport Information

UN Number: Not regulated
UN Proper shipping name: Not classified as dangerous in the meaning of transport regulations.
Transport hazard class(es): No data available
Packing group: No data available
Environmental hazard: No data available
Bulk transport: No data available
Special considerations: No data available

Section 15: Regulatory Information

To the best of our knowledge, this product contains no substances which, at their given concentrations, are considered hazardous by other regulatory agencies. Refer to section 3.

Section 16: Other information

This information is based on our present knowledge. While Abraxis , Inc. believes that the data contained herein are factual and the opinions expressed represent a best effort to present accurate information, the data are not to be taken as a warranty or representation for which Abraxis , Inc. assumes legal responsibility. The information shall not be taken as being all-inclusive and is to be used only as a guide. The data are offered solely for the user's consideration, investigation, and verification. These suggestions should not be confused with either state, municipal, or insurance requirements, or with national safety codes and constitute no warranty. Any use of these data and information must be determined by the user to be in accordance with applicable federal, state, and local regulations.

All materials and mixtures may present unknown hazards and should be used with caution. Since Abraxis , Inc. cannot control the methods, volumes, or conditions of use of this product, Abraxis , Inc. shall not be held liable for any damages or losses resulting from the handling or from contact with the product as described herein. An individual technically qualified to handle potentially hazardous chemicals must supervise the use of this material. This product is sold for research use only. It is not for any human or animal therapeutic or clinical diagnostic use.

Date this SDS was prepared: 5/24/2016
Version: 2
Changes from previous version: Abraxis, LLC changed to Abraxis, Inc.