STUDY TITLE

GREAT STUFF™ POND & STONE WATERFALL FOAM FILLER: EVALUATION OF POTENTIAL PROLONGED TOXICITY TO FRESHWATER FISH

Test Guidelines

OECD, 204 (1984)

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INTRODUCTION

Purpose

The purpose of this study was to determine if application of GREAT STUFF™ Pond & Stone Waterfall Foam Filler in the construction of ornamental ponds has potential to cause adverse effects on freshwater ornamental fish over a prolonged exposure period.

Test Guidelines

The procedure for conducting the 14-day exposure of GREAT STUFF™ Pond & Stone Waterfall Foam Filler to the fathead minnow (*Pimephales promelas*) was adapted from the following test guideline:

OECD Organisation for Economic Cooperation and Development (1984). *OECD Guideline for Testing of Chemicals No. 204,* Fish, Prolonged Toxicity Test: 14-day Study. Adopted 04 April 1984.

Archiving

The data and final report are archived by the Toxicology & Environmental Research and Consulting archivist and stored at The Dow Chemical Company, Midland, Michigan.

Safety

Health and safety exposure data on the test material were reviewed prior to initiation of the study and appropriate safety precautions followed.

MATERIALS AND METHODS

Test Substances

GREAT STUFF™ Pond & Stone Waterfall Foam Filler is a urethane foam product which is dispensed from a pressurized steel canister, using a finger-actuated valve/nozzle. Three 12 oz. canisters containing different developmental foam formulations were received from (Polyurethanes Systems R&D, Willmington, IL). These formulations differed primarily in the colorant additive used, as shown below:

Foam Sample I.D.	Colorant Used
200500675-92A	
200500675-92B	
200500675-92C	

Derivation of Exposure Concentrations

Fish were exposed to concentrations (loadings, g/L) of foam which were approximately 10- and 40-fold higher than a derived minimal loading expected to result from construction of a typical ornamental landscape pond. Kits for do-it-yourself pond construction are sold commercially, and provide materials sufficient for a pond covering 144 square feet (13.4 m^2) and having an average depth of 2.5 feet (0.76 m). This gives a total estimated water volume for a typical pond of approximately 360 cubic feet (10.2 m^3; or 10,200 Liters). In a typical pond construction, a continuous bead of foam (~2 cm diameter) is applied at/above the water line along the entire circumference of the pond liner. The mass of this potentially submerged bead of foam, along with the assumed pond volume above, provides the basis for a calculated minimum foam loading rate (g foam/L water). If the assumed pond is circular in shape, a circumference of 42.7 feet (13 m) is derived; and if the pond is square, a larger circumference of 48 feet (14.6 m) is derived. By dividing this 48 lineal feet (14.6 m) of foam by the 10,200 liter pond volume, a typical foam application rate of approximately 0.005 lineal feet/liter (0.14 lineal cm/liter) was derived. Representative samples of cured foam beads (~2 cm diameter) were shown to have a density of 0.11 ± 0.03 g/lineal cm (mean ± 1 Std. Dev., n = 3). The mathematical product of this density value (0.11 g/lineal cm) and the typical foam application rate (0.14 lineal cm/liter) gives a derived minimum foam loading rate of 0.015 g/L.

Whereas a typical pond construction might utilize up to two 12 oz. cans of foam (*i.e.*, 640 grams applied foam/10,200 Liters = 0.063 g/L), it is also assumed that most of this foam will be applied above the water line, in adhesion of stones and other above-water decorative rock and waterfall features. However, rainfall and periodic watering of landscape vegetation may result in additional potential for washing/leaching of foam components into these ponds. To assess the potential impact from washing/leaching of both submerged and above-water foam applications, foam loadings which are several-fold higher than this derived minimal loading were evaluated in this study. The foam products (~2 cm diameter bead) were applied to the bottom of empty fish aquaria on the basis of foam bead length (see below), and the corresponding weights of uncured foam dispensed from the cans (beginning wt. – final wt. of can for each application to an aquarium) was recorded. Follow their 14-day submersion in water, the foam samples were recovered from the aquaria and dried for 6 hrs. in a vacuum oven at 55 °C. The fish exposure concentrations reported for this study are based on these cured/dry weights of foam. The experimental design and associated foam loadings used to evaluate potential toxicity of the foam are shown in Table 1.

Testing Facilities

Testing was conducted by Toxicology & Environmental Research and Consulting, The Dow Chemical Company, Midland, Michigan.

Laboratory Water

The laboratory dilution water (LDW) was Lake Huron water supplied to The Dow Chemical Company by the City of Midland Water Treatment Plant. The water was obtained from the upper Saginaw Bay of Lake Huron off Whitestone Point and was limed and flocculated with ferric chloride. This water supply is diverted to the laboratory prior to the point at which it is treated for human consumption by the local municipality (City of Midland, Michigan, USA). Before use in the laboratory, the water was sand-filtered, pH-adjusted with gaseous CO_2, carbon-filtered, and UV-irradiated. The LDW was monitored weekly for pH, alkalinity, conductivity, and hardness, and residual chlorine. The hardness (as $CaCO_3$), alkalinity (as $CaCO_3$), and conductivity measured over the four weeks prior to the initiation of the test ranged from 62-72 mg/L, 32-41 mg/L, and 175-191 µmhos/cm, respectively. Residual chlorine concentrations were less than the instrument detection limit of 10 ppb over these entire four weeks. Periodically, the LDW was monitored for total organic carbon (TOC), total suspended solids (TSS), and selected inorganic and organic substances. The most recent analyses for selected inorganic and organic compounds (including TOC and TSS) are presented in Tables 2 and 3, respectively.

Test Organism

The test organism selected for this testing was the fathead minnow, *Pimephales promelas* (Lot # FH060106A), initially obtained from New England Bioassay, Inc., Manchester, Connecticut. This species has been widely recommended and accepted over several decades for use in standardized aquatic toxicity testing, such as the OECD Guideline 204 (OECD, 1984). The fathead minnow belongs to the *Cyprinidae* (carp) family, as do most of the freshwater fish which are maintained in ornamental ponds. Adult male fish (~10-11 months old) of the same lot and age were used as the test organisms (see Table 4 for typical holding conditions). The fish were acclimated to test conditions for a period of greater than 14 days prior to testing. The batch of fish used for testing exhibited < 5% mortality during the 48-hours prior to beginning of the foam exposures.

A representative sample (n = 10, equivalent to the number of fish exposed per test level) of the fish from the lot used for testing measured on day 0 had a mean wet (blotted dry) weight of 1.7 g (range: 1.3 to 2.5 g) and a mean total length of 5.7 cm (range: 5.2 to 6.3 cm), which were considered representative of the length and weight of all fish used in the study. The length of the largest fish was no more than twice that of the shortest fish. Fish were euthanized with tricaine methanesulfonate prior to the length and weight measurements. The biological loading rate, calculated as [(mean fish weight of 1.7 g × 10 fish per vessel)/15 L of test solution per vessel], was 1.1 g fish/L. These size and biomass loading parameters fell within ranges specified in the OECD test guideline (OECD, 1984).

EXPERIMENTAL

Study Design

Duration:	14-days
Exposure Scenario:	Static, with renewal after 7 days
Test Concentrations:	0.15 and 0.63 g dry foam/L for each product tested and a water control. Equivalent to approximately 10 and 40 times the expected minimum foam loading for a typical pond application.
Carrier Solvent:	Not applicable
Test Temperature:	25 ± 1°C. Measured every Monday, Wednesday, and Friday from all test vessels containing fish. Continuously measured from one vessel over the entire duration of exposure.
Aeration:	Continuous mild aeration in each test vessel throughout the study.
Dissolved Oxygen:	Measured every Monday, Wednesday, and Friday from all test vessels containing fish.
pH:	Measured every Monday, Wednesday, and Friday from all test vessels containing fish.
Photoperiod:	16-hour light/8-hour dark
Light Intensity:	Measured at each vessel location at test initiation.

Test Vessel:	Approximately 20 L (~20 x 41 x 25 cm) glass aquarium. All aquaria were loosely covered and uniquely labeled for identification purposes.
Vessel Replicates:	Duplicate aquaria (replicates A and B) prepared at same time point, with only one replicate initially containing fish. The fish were transferred on exposure day 7 from replicate A to replicate B vessels, which contained the same nominal foam loadings. This renewal was performed in an effort to maintain water quality conditions and consistent exposure concentrations over the long-term test.
Test Solution Volume:	15 L
Loading:	10 fish/15 L
Test Organism Age:	Adults (10-11 months old)
Feeding:	Daily, Frozen (thawed) adult brine shrimp (Brine Shrimp Direct, Odgen, Utah. Cat# FBSFKG210). Fed approximately 4% fish body wet (blotted dry) weight (measured at test initiation) per day.
Test Location:	Static water trough
Observations:	Daily for mortality, abnormal behavior, and changes in appearance or physiology, if present.
Measured Effect:	Mortality, sublethal effects
Endpoints:	Threshold level of lethal effect (the lowest loading of the test substance in the test solution at which the substance has a lethal effect) Threshold level of observed effect (the lowest loading of the test substance in the test solution at which the substance is observed to have an effect other than lethal on a significant number of test fish) NOELR (no-observed-effect loading rate) or the highest loading of a test substance at which no statistically significant lethal or other effect is observed.

Acceptance Criterion: ≤ 10% control mortality

Test Solution Preparation

The three foam samples were directly applied on the inside bottoms of identical 20 L glass aquaria. Prior to application, the aquaria were thoroughly washed/rinsed, and then misted with MilliQ water to promote reaction/adhesion of the foam on the glass surface. Each canister was vigorously shaken for several minutes to homogenize contents, and several grams of product were dispensed and discarded to ensure that a representative product was applied in the aquaria. The foam was applied along a longitudinal center line of the aquarium bottom, in a single continuous bead of approximately 2 cm diameter and either 7 lineal cm or parallel beads of 71 lineal cm total (*i.e.*, 2 x ~35 cm beads). It was noted that foam density decreased with increased bead length, so that these approximate lineal foam loadings did not correlate exactly with their associated measured gravimetric foam loadings. The gravimetric foam loadings are referred to as "10X" and "40X" in the experimental design (Table 1), and were approximately 10- and 40-fold higher than the derived minimal loading rate of 0.015 g/L. The gross weight of the foam dispensing can was recorded before and after each application, to determine the actual loading of wet/uncured foam in each aquarium. Following application, the aquaria were loosely covered, and foam was allowed to cure for 24 hrs. After this curing step, the aquaria were rinsed, and then filled, with laboratory dilution water (LDW). In each aquarium, two semi-cylindrical spawning tiles (stainless steel) were placed (straddled) over the foam. These were added to prevent the foam from floating to the surface, should it become dislodged from the aquarium bottom. Also, because the fathead minnows tend to spend most of their time within these tiles, the fish were kept in close proximity to the foam over the entire duration of the study. Fish were added to the "A" replicate aquaria, after the water temperature and dissolved oxygen concentrations had attained the desired test conditions. After the initial seven days of exposure in the "A" aquaria, the fish were transferred to their corresponding "B" aquaria and remained there for an additional seven days.

Sampling and Analyses

The "B" replicate aquaria containing applications of the three foam products were equilibrated with LDW in absence of fish for seven days. Water samples from these aquaria were collected, filtered, and analyzed for dissolved organic carbon (DOC) after three and seven days to determine the extent to which foam components were dissolved in the exposure medium, and whether the concentrations of these dissolved components were constant over this period. Analyses were not performed beyond seven days, or for aquaria which contained fish, as these DOC results would include contributions from food residues and fish excrement.

The DOC analyses were performed using a Shimadzu model TOC-V carbon analyzer, which determines total carbon (TC) by combustion, inorganic carbon (IC) by acidification, and total organic carbon (TOC) by difference of TC and IC. Prior to analysis, the water samples were filtered (0.45 µm nylon syringe filters), where the filters were pre-rinsed with MilliQ water and the first 5 mL of sample filtrate was discarded. For these analyses, DOC is defined as the TOC result for samples which have been filtered in such a manner.

RESULTS AND DISCUSSION

Test Conditions

A summary of the test conditions recorded over the 14-day fish exposure is provided in Table 5. Individual measurements for dissolved oxygen, temperature, pH, and light intensity are reported in Appendix A. Dissolved oxygen levels ranged from 6.8-7.9 mg/L (84-98% oxygen saturation) over the 14-day exposure period. Temperature, as measured daily from the individual test vessels and continuously measured from one surrogate test vessel, remained at 25 °C throughout the study. The pH ranged from 7.1-7.7 and the light intensity ranged from 701-1263 lux. Therefore, exposure conditions of temperature, pH, dissolved oxygen, and lighting were maintained within the required ranges over the entire 14-day exposure.

Biological Data

A summary of the biological observations recorded over the course of the 14-day fish exposures of are presented in Table 6. The fish were observed daily for sublethal effects (*e.g.,* lethargy, loss of equilibrium) and mortality. Over the 14-day exposure period, no sublethal effects were observed in any of the treatments and only a single mortality was observed. This single mortality was observed on day 10 for the 40X- treatment with foam sample "C"), and was believed to be incidental and non-treatment related, since no other fish at this test level exhibited signs of stress or sublethal effects during the test. In addition, the incidence of mortality in this treatment (10%) did not exceed the accepted incidence of mortality in the control exposure of ≤ 10%, which is a test validation criterion specified in the OECD guideline (OECD, 1984).

Therefore, the threshold level of lethal effect, threshold level of observed effect, and NOEC for each product tested was > 0.63 g/L, which is approximately 40 times the expected foam loading rate expected to result from use of the foam in construction of a typical ornamental pond.

The foam loading rates evaluated in this study are expected to represent loading rates which are much higher than would result from typical construction of an ornamental pond. It should be noted that the highest (40x) loading rate is also approximately equivalent to that which would occur from dispensing two 12-ounce cans of the foam product (approx. 640 grams) at or below the water level of a typical 10,200 L pond (*i.e.*, 0.63 g foam/L). No adverse effects were noted with fish which were exposed to this foam loading rate of 0.63 g/L, thus demonstrating a large margin of safety for freshwater ornamental fish which might be maintained in ponds constructed with the GREAT STUFF™ Pond & Stone Waterfall Foam Filler.

Analytical Results

The results of DOC analyses recorded for the "B" replicate test solutions after three and seven days contact with the three foam samples are summarized in Table 7. For these analyses, the DOC concentration measured in the Control water was subtracted from that measured in the foam-exposed water for each sampling interval. This allowed a determination and comparison of the concentration of DOC attributed to components which had leached from each of the three foam formulations. After three days contact with the foam, the test solutions for "10X" loading of the three foams contained from 0.2 to 0.8 mg/L foam-associated DOC, whereas the "40X" loadings resulted in 1.1 to 1.4 mg/L foam-associated DOC. Continued contact of the foam with water for an additional four days resulted in only slight increases in foam-associated DOC. After seven days of contact with water, the foam-associated DOC resulting from the "10X" loading ranged from 0.6 to 0.9 mg/L for the three foam samples, and that from the "40X" loading ranged from 1.1 to 2.1 mg/L. None of the three foam products was consistently associated with the lowest or highest foam-associated DOC concentration, indicating that the three foams products did not have notable differences in their water-extractable components (Table 7). The concentrations of foam-associated DOC were increased with increased foam loading, but these increases were neither uniform nor directly proportional to the 4-fold difference in loading (g/L) of the three foam formulations. This foam-associated DOC increased from between 1.2- and 6.5-fold with the 4-fold increase in foam loadings (Table 7). Although these DOC concentrations are close to the detection limit for the analysis, they suggest that the component(s) which contribute to this foam-associated DOC have limited water solubility.

Assessment of Foam Components

The apparent lack of toxicity to fish is consistent with the low water solubility/extractability and acute toxicity properties of the known components of the GREAT STUFF™ Pond & Stone Waterfall Foam Filler. These components, and their associated measured or predicted water solubilities and median acute toxicity concentrations (*i.e.*, LC_{50}) to fish are summarized in Table 8. The principal component of this product, polymeric methylenediphenyldiisocyanate (polymeric MDI) is reactive with water, and this rapid hydrolysis reaction results primarily in formation of solid, cross-linked polyureas. In laboratory tests of acute toxicity to fish, polymeric MDI exhibits typical 96 hr. LC_{50} values of > 1,000 mg/L (EC, 2000). In a pond mesocosm study, polymeric MDI loadings of 1,000 and 10,000 mg/L appeared to have no direct toxic effect on rainbow trout over a 112-day exposure period (Heimbach *et al.*, 1996). However, weight loss and mortality in these fish were observed with prolonged containment of the fish in these ponds, which was linked to food deprivation associated with smothering of aquatic plants and invertebrates (cladocerans) by the mass of solid polyureas formed by the hydrolyzed polymeric MDI (Heimbach *et al.*, 1999). The polyether polyol components, having moderate water solubility, are also shown to be practically non-toxic to aquatic organisms (EC, 2000). Since the polymeric MDI component is present at a concentration in excess of that required for reaction with the hydroxyl groups of the polyol and colorant components, these components will be fully (covalently) bound into the foam matrix upon its curing.

The blowing agents (dimethyl ether, isobutene, propane) are expected to largely volatilize during application and curing of the foam, as evidenced from the differences in recorded weights of the applied and cured/dried foams (Table 1). The differences between the mean applied and cured weights are approximately 14 and 17 % for the 10X and 40X foam loadings, respectively; while the blowing agents comprise approximately 14 % wt. of the typical foam formulation (Table 8). The medium-chain (C_{14-17}) chlorinated paraffin component is not acutely toxic to fish at the limit of its water solubility. The flame retardant), while moderately soluble in water, is regarded as moderately to essentially non-toxic to fish (EC, 2000). Similarly, the atalyst component) is expected to be essentially non-toxic, despite its miscibility in water (U.S. EPA, 2004).

Finally, the GREAT STUFF™ Pond & Stone Waterfall Foam Filler forms a rigid, closed-cell foam which is encased in a continuous outer "skin" upon curing. These properties will restrict intrusion of water into the foam, and also mitigate leaching of any unbound components out of the foam. Therefore, the results of this study are consistent with the expected water solubility/extractability and ecotoxicity of the foam components.

CONCLUSIONS

Based on the absence of any treatment-related mortality or sublethal effects over a 14-day exposure to fathead minnows, the threshold level of lethal effect, threshold level of observed effect, and NOEC for three different formulations of GREAT STUFF™ Pond & Stone Waterfall Foam Filler was empirically determined to be greater than the highest foam loading tested of 0.63 g foam/L. This loading rate is equivalent to approximately 40 times the minimum foam loading expected to result from its use in construction of a typical ornamental pond. Based on results of this assessment, the use of these three formulations of GREAT STUFF™ Pond & Stone Waterfall Foam Filler in construction of ornamental ponds is regarded as having low potential for causing acute and/or prolonged toxicity to freshwater fish maintained in these ponds.

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TABLES AND FIGURES

Table 1. Experimental Design and Foam Loadings Used to Evaluate Potential Prolonged Toxicity of GREAT STUFF™ Pond & Stone Waterfall Foam Filler to Fathead Minnows.

Foam Product Identity	**Aquarium I.D. (Loading-Foam-Replicate)**	**Foam Loading**	
		Applied Foam Conc. (g/L)*	**Exposed/Dry Foam Conc. (g/L)****
-	Control-A	-	-
-	Control-B	-	-
200500675-92A	10X-A-A	0.22	0.19
	10X-A-B	0.18	0.16
	40X-A-A	0.68	0.56
	40X-A-B	0.65	0.78
200500675-92B	10X-B-A	0.15	0.12
	10X-B-B	0.18	0.15
	40X-B-A	0.73	0.61
	40X-B-B	0.71	0.58
200500675-92C	10X-C-A	0.17	0.15
	10X-C-B	0.18	0.15
	40X-C-A	0.64	0.54
	40X-C-B	0.81	0.70
Mean (± 1 Std. Dev.) for 10X Loading		0.18 ± 0.02	0.15 ± 0.02
Mean (± 1 Std. Dev.) for 40X Loading		0.73 ± 0.06	0.63 ± 0.09

*based on difference of applicator can weight before and after application to aquarium, 15 L total water volume
**based on dried weight of foam beads recovered from aquaria after 14 days immersion in water

Table 2. Results for Analyses of Selected Inorganic Compounds in the Laboratory Dilution Water (LDW).

	[a]Laboratory Dilution Water (Standard)
Analyte	μg/L
Aluminum	**3**
Arsenic	[b]BDL(0.7)
Boron	BDL(100)
Beryllium	BDL(0.1)
Calcium	**14,600**
Cadmium	BDL(0.1)
Chromium	**4**
Cobalt	BDL(0.1)
Copper	BDL(0.1)
Iron	**20**
Lead	**0.5**
Magnesium	**6,440**
Manganese	BDL(0.1)
Mercury	BDL(0.01)
Molybdenum	**2.5**
Nickel	**10.4**
Potassium	**410**
Selenium	BDL(0.6)
Silver	BDL(7)
Sodium	**4,100**
Zinc	BDL(0.1)
Ammonia	BDL(100)
Bromide	BDL(1000)
Chloride	**13,470**
Cyanide	BDL(1)
Fluoride	**100**
Iodide	BDL(1,000)
Nitrate	**290**
Residual Chlorine	BDL(20)
Sulfate	**16,000**
Sulfide	BDL(1000)
TSS	**3000**
[c]TOC	**380**

[a]Sampled on 7/24/06. Analysis by A&L Great Lakes Laboratories, Inc., Fort Wayne, Indiana. (Report No. F06206-8011).
[b]BDL = Below detection level, indicated in parentheses, of method utilized.
[c]As determined by C. Hales, The Dow Chemical Company, Date Sampled 8/18/06.

Table 3. Results of Analysis for Selected Organic Substances and Pesticides in the Laboratory Dilution Water (LDW).

Analyte	µg/L	Analyte	µg/L
POLYCYCLIC AROMATIC HYDROCARBONS		ORGANOCHLORINE PESTICIDES	
Acenaphthene	<DL3	Aldrin	<DL1
Acenaphthylene	<DL3	a-BHC	<DL1
Anthracene	<DL3	b-BHC	<DL1
Benzo(a)anthracene	<DL3	g-BHC (Lindane)	<DL1
Benzo(b)fluoranthene	<DL3	d-BHC	<DL1
Benzo(k)fluoranthene	<DL3	Chlordane	<DL2
Benzo(g,h,i)perylene	<DL3	p,p'-DDD	<DL2
Benzo(a)pyrene	<DL3	p,p'-DDE	<DL2
Chrysene	<DL3	p,p'-DDT	<DL2
Dibenzo(a,h)anthracene	<DL3	Dieldrin	<DL2
Fluoranthene	<DL3	Endosulfan I	<DL1
Fluorene	<DL3	Endosulfan II	<DL2
Ideno(1,2,3-c,d)pyrene	<DL3	Endosulfan Sulfate	<DL2
Phenanthrene	<DL3	Endrin	<DL2
Pyrene	<DL3	Endrin Aldehyde	<DL2
		Heptachlor	<DL1
ORGANOPHOSPHATE INSECTICIDES		Heptachlor Epoxide	<DL1
Diazinon	<DL2	Hexachlorobenzene (HCB)	<DL1
Ethyl Parathion	<DL2	Methoxychlor	<DL4
Malathion	<DL2	Mirex	<DL4
Methyl Parathion	<DL2	Toxaphene	<DL5
CHLOROPHENOXY HERBICIDES			
Dicamba	<DL1		
2,4-D	<DL3		
2,4,5-T	<DL1		
2,4,5-TP (Silvex)	<DL1		

Sampled on 7/24/06. Analysis by A&L Great Lakes Laboratories, Inc., Fort Wayne, Indiana. (Report No. F06206-8011)

<DL1 = Below detection limit of 0.25 µg/Liter
<DL2 = Below detection limit of 0.5 µg/Liter
<DL3 = Below detection limit of 1 µg/Liter
<DL4 = Below detection limit of 2.5 µg/Liter
<DL5 = Below detection limit of 5 µg/Liter

Table 4. Summary of Typical Holding Conditions for Fathead Minnows Used in Evaluating Potential Prolonged Toxicity of GREAT STUFF™ Pond & Stone Waterfall Foam Filler.

Test Species:	*Pimephales promelas*
Temperature:	25 ± 2ºC
Photoperiod:	16-hour light/8-hour dark
Water:	Laboratory dilution water (LDW)
Holding Vessels:	230 L or 430 L (stainless steel or plexiglass) tanks with continuous water flow.
Feed:	Frozen adult brine shrimp (Brine Shrimp Direct, Odgen, Utah, Cat#FBSFKG210) *ad libitum* daily.

Table 5. Summary of Test Conditions Employed in Evaluating Potential Prolonged Toxicity of GREAT STUFF™ Pond & Stone Waterfall Foam Filler to Fathead Minnows.

Light intensity range (lux)	701 - 1263
Temperature range (°C):	
From individual test vessels	25 °C
Continuous monitoring:	25 °C
pH range	7.1 - 7.7
Dissolved oxygen range (mg/L)	6.8 - 7.9
Percent oxygen saturation range (%)	84 - 98
Average (range, grams) fish weight *	1.7 (1.3 - 2.5)
Average (range, cm) fish length *	5.7 (5.2 - 6.3)
Number of fish per replicate test vessel	10
Test vessel fill volume (L)	15
Instantaneous biological loading rate (g-fish/L) *	1.1
Daily Feeding of ~4% fish wet weight (mL of brine shrimp/vessel)	0.5

*Values based on day 0 measurements obtained from a representative sample (N = 10, equivalent to the number of fish per test level) of the fish from the lot used for the test.

Table 6. Summary of Biological Observations Recorded During 14-day Exposure of Fathead Minnows

GREAT STUFF™ Pond & Stone Waterfall Foam Filler	Observation Day													
	1	2	3	4	5	6	7[a]	8	9	10	11	12	13	14
Water Control A	10N	10N	10N	10N	10N	10N	10N							
Water Control B								10N	10N	10N	10N	10N	10N	10N
10X-A-A	10N	10N	10N	10N	10N	10N	10N							
10X-A-B								10N	10N	10N	10N	10N	10N	10N
40X-A-A	10N	10N	10N	10N	10N	10N	10N							
40X-A-B								10N	10N	10N	10N	10N	10N	10N
10X-B-A	10N	10N	10N	10N	10N	10N	10N							
10X-B-B								10N	10N	10N	10N	10N	10N	10N
40X-B-A	10N	10N	10N	10N	10N	10N	10N							
40X-B-B								10N	10N	10N	10N	10N	10N	10N
10X-C-A	10N	10N	10N	10N	10N	10N	10N							
10X-C-B								10N	10N	10N	10N	10N	10N	10N
40X-C-A	10N	10N	10N	10N	10N	10N	10N							
40X-C-B								10N	10N	9N, 1D	9N	9N	9N	9N
Results (grams GREAT STUFF™ Pond & Stone Waterfall Foam Filler /L)														
Threshold Level of Lethal Effect	> 0.63 g/L													
Threshold Level of Observed Effect	> 0.63 g/L													
NOEC	> 0.63 g/L													

[a] Fish transferred from replicate A tank to Replicate B tank of the same test concentration on exposure day 7.

Table 7. Results of Dissolved Organic Carbon (DOC) Analyses of Fish Exposure Solutions Following Three and Seven Days Contact with GREAT STUFF™ Pond & Stone Waterfall Foam Filler at Loadings of Approximately 0.15 (10X) and 0.63 (40X) g/L.

Foam Product I.D.	Aquarium I.D. (Loading-Foam-Replicate)	Control-Corrected DOC (mg/L)	
		Day 3	**Day 7**
200500675-92A	10X-A-B	0.8	0.9
	40X-A-B	1.4	1.1
200500675-92B	10X-B-B	0.5	0.8
	40X-B-B	1.1	1.3
200500675-92C	10X-C-B	0.2	0.6
	40X-C-B	1.3	2.1

Table 8. Representative Composition of Single-Component Foam (GREAT STUFF™ Pond & Stone Waterfall Foam Filler) and Associated Water Solubility and Acute Fish Toxicity Properties.

Component (CAS Registry No.)	% Wt. of Composition	Water Solubility (mg/L)	Fish 96 hr. LC_{50} (mg/L)	Reference
Polymeric MDI (9016-87-9)		Insoluble (reacts)	> 1,000	EC (2000)
Polyol		Miscible	> 100	EC (2000)
Polyol		10,000	> 100 – > 1,000	EC (2000)
[redacted]		1,500	30 – 180	EC (2000)
Chlorinated paraffin (C_{14-17}) (63449-39-8)		< 0.005	> 100	EC (2000)
		Miscible	8,800*	U.S. EPA (2004)
(proprietary composition)		Miscible	Unknown	-
Blowing agent: Dimethyl ether (115-10-6) Propane (74-98-6) Isobutane (75-28-5)		 328,000 62 49	 > 4,000 > 1,000 > 1,000	EC (2000)
(proprietary compositions)		Miscible	Unknown	

*predicted value; measured value not available

APPENDICES

Appendix A1. Individual Dissolved Oxygen Measurements

GREAT STUFF™ Pond & Stone Waterfall Foam Filler	Replicate	Dissolved Oxygen (mg/L)						
		Day 0	Day 3	Day 5	Day 7[a]	Day 10	Day 12	Day 14
Water Control A	A	7.3	7.8	7.4	7.2			
Water Control B	B				7.9	7.2	7.3	7.8
1X-A-A	A	7.4	7.7	7.4	7.2			
1X-A-B	B				7.8	6.9	6.8	7.7
10X-A-B	A	7.3	7.2	7.5	7.1			
10X-A-B	B				7.8	7.0	7.0	7.7
1X-B-A	A	7.3	7.6	7.8	7.2			
1X-B-B	B				7.8	7.2	7.4	7.4
10X-B-B	A	7.0	7.5	7.3	7.2			
10X-B-B	B				7.8	6.8	7.2	7.2
1X-C-A	A	7.2	7.3	7.0	7.5			
1X-C-B	B				7.7	7.0	7.0	7.1
10X-C-B	A	7.2	7.4	7.1	7.4			
10X-C-B	B				7.7	7.3	7.3	7.4
Minimum (% Oxygen Saturation[b]):		6.8 (84%)						
Maximum (% Oxygen Saturation):		7.9 (98%)						

[a] Fish transferred from replicate A vessel to Replicate B vessel of the same test concentration on exposure day 7.

[b] Percent air saturation = measured DO value/Theoretical DO value for air-saturated water (8.1 mg/L at target test temperature of 25°C).

Appendix A2. Individual pH Measurements

GREAT STUFF™ Pond & Stone Waterfall Foam Filler	Replicate	pH						
		Day 0	Day 3	Day 5	Day 7[a]	Day 10	Day 12	Day 14
Water Control A	A	7.2	7.2	7.3	7.2			
Water Control B	B				7.5	7.5	7.4	7.4
1X-A-A	A	7.3	7.4	7.3	7.4			
1X-A-B	B				7.6	7.4	7.3	7.5
10X-A-B	A	7.3	7.3	7.3	7.4			
10X-A-B	B				7.6	7.4	7.4	7.6
1X-B-A	A	7.2	7.3	7.4	7.5			
1X-B-B	B				7.7	7.5	7.5	7.5
10X-B-B	A	7.1	7.4	7.4	7.5			
10X-B-B	B				7.6	7.4	7.4	7.4
1X-C-A	A	7.2	7.2	7.4	7.7			
1X-C-B	B				7.7	7.4	7.4	7.3
10X-C-B	A	7.3	7.4	7.5	7.7			
10X-C-B	B				7.7	7.5	7.5	7.4
Minimum:		7.1						
Maximum:		7.7						

[a] Fish transferred from replicate A vessel to Replicate B vessel of the same test concentration on exposure day 7.

Appendix A3. Individual Temperature Measurements

GREAT STUFF™ Pond & Stone Waterfall Foam Filler	Replicate	Temperature[a,b] (°C)						
		Day 0	Day 3	Day 5	Day 7[c]	Day 10	Day 12	Day 14
Water Control A	A	25	25	25	25			
Water Control B	B				25	25	25	25
1X-A-A	A	25	25	25	25			
1X-A-B	B				25	25	25	25
10X-A-B	A	25	25	25	25			
10X-A-B	B				25	25	25	25
1X-B-A	A	25	25	25	25			
1X-B-B	B				25	25	25	25
10X-B-B	A	25	25	25	25			
10X-B-B	B				25	25	25	25
1X-C-A	A	25	25	25	25			
1X-C-B	B				25	25	25	25
10X-C-B	A	25	25	25	25			
10X-C-B	B				25	25	25	25
	Minimum:	25						
	Maximum:	25						

[a] Temperature measurements were recorded to the nearest 0.1 degree and reported to the nearest 1 degree (≥ 0.5 degrees round up; ≤ 0.4 degrees round down).

[b] Temperature continuously monitored in one surrogate test vessel, remained constant at 25 °C during the exposure period.

[c] Fish transferred from replicate A vessel to Replicate B vessel of the same test concentration on exposure day 7.

Appendix A4. Individual Light Intensity Measurements

GREAT STUFF™ Pond & Stone Waterfall Foam Filler	Replicate	Light Intensity (Lux)
		Day 0
Water Control A	A	1042
Water Control B	B	1178
1X-A-A	A	1154
1X-A-B	B	1233
10X-A-B	A	1263
10X-A-B	B	1125
1X-B-A	A	1211
1X-B-B	B	1116
10X-B-B	A	1097
10X-B-B	B	1047
1X-C-A	A	800
1X-C-B	B	939
10X-C-B	A	937
10X-C-B	B	701
	Minimum:	701
	Maximum:	1263