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Ecotoxicology and Environmental Safety 57 (2004) 375–382
Ecotoxicology and Environmental Safety
http://www.elsevier.com/locate/ecoenv

Evaluating the toxicity of Triton X-100 to protozoan, fish, and mammalian cells using fluorescent dyes as indicators of cell viability

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Received 3 September 2002; received in revised form 1 May 2003; accepted 2 May 2003

Abstract

Three viability assays using fluorescent dyes effectively detected a loss of viability in cultures of three mammalian cell lines (H4IIE, Caco2, and HepG-2), two fish cell lines (RTgill-W1 and RTL-W1), and a ciliated protozoan, *Tetrahymena thermophila,* after exposure to Triton X-100, used as a model toxicant. The dyes were Alamar Blue (AB), neutral red (NR), and propidium iodide, which respectively monitored energy metabolism, lysosomal activity, and membrane integrity. A fourth fluorescent dye, 5-carboxyfluorescein diacetate acetoxymethyl ester, was problematic. For 2-h Triton X-100 exposures, mammalian cell lines were as susceptible as piscine cell lines, whereas *T. thermophila* was approximately twofold less sensitive as detected with AB and NR. Despite being less sensitive, cytotoxicity tests on *T. thermophila* could be done in spring water, which means that unlike animal cells they could be directly exposed to most industrial effluents without osmolality adjustments. Therefore, *T. thermophila* could be a useful complement to animal cells as alternatives to fish in toxicity testing.

Keywords: Fluorescent indicator dyes; Cell viability assay; Surfactant; *Tetrahymena*; Fish cells; Mammalian cells; Ciliate

1. Introduction

Fish cell cultures are being considered as an alternative to whole fish in the testing of industrial effluents for toxicants (Ahne, 1985; Castaño et al., 1996; Dayeh et al., 2002). They promise both to fulfil a societal desire, which is to see fewer animals used in toxicity testing, and to yield results more rapidly and more cost effectively than conventional tests with whole fish. Furthermore, cultures of fish cell lines have an advantage over primary cultures in not requiring more fish and have an advantage over mammalian cells in being a more reasonable substitute for fish.

A problem with using cultures of cells from fish or any other vertebrate is the necessity of getting the effluent in a form suitable for presentation to cells in culture. Direct addition is usually not possible because commonly the effluent is hypotonic and has microbes. The simplest sample preparation so far involves dissolving the salts of a basal medium for animal cells into the effluent to get the correct osmolality and filtering the effluent prior to adding it to fish cells to remove microbial contamination (Dayeh et al., 2002). Treated in this way, cultures of a rainbow trout gill cell line (RTgill-W1) identified the only effluent sample that was toxic to rainbow trout in effluents from a paper mill. Testing would be even more convenient if no sample preparation were necessary and only the cells had to be added to the effluent. This could be done with a unicellular eukaryote, such as the ciliated protozoan, *Tetrahymena thermophila* (Gilron and Lynn, 1996).

The use of cells as alternatives to animals in toxicity testing requires rapid and sensitive cell viability assays. Viability tests in microwell plates using fluorescent dyes have several desirable features. First, many indicator dyes have become commercially available, increasing the range of cellular parameters that potentially can be monitored. For example, the neutral red (NR) assay measures the activity of lysosomes, with the principle

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0147-6513/03/$ - see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/S0147-6513(03)00083-6

being that only the lysosomes of viable cells will fluoresce after accumulating the dye (Essig-Marcello and van Buskirk, 1990). Propidium iodide (PI) stains double-stranded nucleic acids and is excluded by living cells; as a result cell fluorescence indicates an impairment of plasma membrane function (Wrobel et al., 1996). Alamar Blue (AB) is a commercial preparation of the dye resazurin and is reduced to a fluorescent form by viable cells, with a diminishment in reduction indicating an impairment of cellular metabolism (O'Brien et al., 2000). Finally, 5-carboxyfluorescein diacetate acetoxymethyl ester (CFDA-AM) diffuses into cells and is converted by the nonspecific esterases of living cells from a nonpolar, nonfluorescent dye into a polar, fluorescent dye (5-carboxyfluorescein, CF), which diffuses out of cells slowly. A decline in fluorescence readings is interpreted as a loss of plasma membrane integrity (Ganassin et al., 2000). Using these dyes in microwells conserves resources and reduces the amount of valuable test agents and the number of cells used. As well, some dyes, such as AB and CFDA-AM, can be used together to monitor individual cultures (Schirmer et al., 1997). Finally, linking of fluorometric multiwell plate readers to computers has made measurement and analysis rapid and easy (O'Connor et al., 1991).

Tetrahymena is increasingly being used in toxicology testing (Sauvant et al., 1999). The most common endpoint has been an impairment of cell growth rather than a loss of cell viability (Larsen et al., 1997; Schultz, 1997; Sinks and Schultz, 2001; Slabbert and Morgan, 1982). Only a few toxicity studies with various species of *Tetrahymena* have measured cell viability as an endpoint using the dyes NR and 3-(4,5-dimethylthizol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (Dias et al., 1999; Wang et al., 2000). These were monitored spectrophotometrically. Thus, fluorescent approaches appear not to have been investigated with *Tetrahymena*.

Available background information makes surfactants suitable toxicants for evaluating cell viability assays. As humans can be exposed to surfactants in cosmetic, pharmaceutical, and dermatological formulations, they have been studied frequently for their effect on the viability of human cells (Jelinek and Klocking, 1998). Fish cells have been studied less frequently, but fish can be exposed to surfactants through their release by several industries, including the pulp and paper and textile industries (Lee, 1999). The actions of surfactants on *Tetrahymena* have also been studied (Nicolau et al., 1999). In our study, Triton X-100 has been used as a model toxicant and surfactant: (A) to identify the fluorometric assays most suitable for measuring the viability of animal cells after surfactant exposure, (B) to determine the applicability of the fluorometric viability assays to *T. thermophila*, and (C) to compare the sensitivity of *T. thermophila* with piscine and mammalian cells. The results suggest that viability of *T. thermophila* in response to the toxicity of surfactant-containing industrial effluent can be monitored using fluorescence assays.

2. Materials and methods

2.1. Fish and mammalian cell cultures

2.1.1. Fish cell lines

The fish cell lines RTL-W1 from the liver (Lee et al., 1993) and RTgill-W1 (ATCC Accession No. CRL-2523) from the gill (Bols et al., 1994) of rainbow trout (*Oncorhynchus mykiss*) were used for assays. Cells were cultured routinely in 75-cm^2 culture flasks at 20°C in Leibovitz's L-15 culture medium (Gibco BRL, Burlington, ON, Canada), which was supplemented with fetal bovine serum (FBS, Sigma) (10% for RTgill-W1 and 5% for RTL-W1) and 2% penicillin–streptomycin (100 μg/mL streptomycin, 100 IU/mL penicillin; Gibco BRL). Culture supplies and subcultivation procedures were as previously described (Bols and Lee, 1994; Schirmer et al., 1994). Prior to exposure, cells were plated in 96-well tissue culture plates (Falcon; Becton–Dickinson, Franklin Lakes, NY, USA) at a density of 5×10^4 cells per 200 μL L-15 with FBS and grown to confluency.

2.1.2. Mammalian cell lines

The mammalian cell lines were the rat liver hepatoma cell line H4IIE (ATCC Accession No. CRL-1600) and two human cell lines, Caco2, a colon adenocarcinoma cell line (ATCC Accession No. HTB-37), and HepG2, a liver hepatoma cell line (ATCC Accession No. CRL-11997). Cells were routinely cultured in 75-cm^2 vented culture flasks at 37°C in a humidified 5% CO_2–95% atmosphere. H4IIE cells were cultured in Dulbecco's medium supplemented with 10% FBS, 0.1% gentamycin sulfate (50 mg/mL), 2% L-glutamine, 2% minimal essential medium (MEM) nonessential amino acids, 3% MEM amino acids, and 1.5% MEM vitamins (all components from Sigma). Both Caco2 and HepG2 cells were cultured in Dulbecco's MEM modified with Earle's salts supplemented with 10% FBS, 0.1% gentamycin sulfate (50 mg/mL), 1% L-glutamine, 2% MEM nonessential amino acids, and 0.2% sodium pyruvate (55 mg/mL). Prior to exposure, cells were plated in 96-well tissue culture plates (Falcon) at a density of 5×10^4 cells per 200 μL L-15 with FBS and grown to confluency.

2.2. T. thermophila cultures

The ciliate *T. thermophila* (Oligohymenophorea; Tetrahymenidae) was routinely cultured axenically in 10 mL of proteose peptone yeast extract medium

(PPYE) as described in Gilron et al. (1999). Approximately 2–3 mL of the existing culture was routinely transferred every 2–3 weeks into 10 mL of fresh, sterile PPYE medium to keep a supply culture of *T. thermophila* on hand. To prepare the cultures for testing, an adaptation of the protocol from Gilron et al. (1999) was used. Briefly, from the stock culture, 1 mL was transferred into 10 mL of sterile PPYE and grown for 24 h. Then, the 10 mL culture was transferred to 50 mL of sterile PPYE in a 250-mL Erlenmeyer flask and grown on an orbital shaker (50 rpm) for 1–2 days. The culture was then centrifuged and washed three times with spring water (pH 7.32; water hardness 235 ppm measured as calcium hardness; and conductivity 840 μS/cm) (Aberfoyle Springs, Aberfoyle, ON, Canada). Cells were centrifuged again and resuspended in 10 mL of spring water. Cells were counted using a Coulter Z2 particle counter and adjusted to a cell density of 500,000 cells/mL (±10%) using spring water.

2.3. Triton X-100 treatments

2.3.1. Exposure of fish and mammalian cell lines

Upon confluency, the cultures were exposed to increasing concentrations of Triton X-100 (*t*-octylphenoxypolyethoxyethanol). A stock solution of 1 mg/mL Triton X-100 (Sigma) was prepared and serially diluted in the modified basal medium L-15/ex (Schirmer et al., 1997).

Exposure was initiated by removing the culture medium from confluent cells. The cells were washed once with L-15/ex. Then, 200 μL/well of the serial dilutions of Triton X-100 in L-15/ex was added. The cells were exposed at their routine culturing temperatures of 20°C and 37°C for the fish and the mammalian cell lines, respectively. After 2 h of exposure to Triton X-100, cultures were assessed as described under cell viability assays.

2.3.2. Exposure of T. thermophila

A stock solution of 1 mg/mL Triton X-100 in spring water was serially diluted in spring water with concentrations ranging from 0 (control) to 400 μg/mL. Exposure of *T. thermophila* was conducted in microcentrifuge tubes. First, *T. thermophila* was added in 1 mL of spring water to the microcentrifuge tubes at a density of 500,000 cells/mL. After the pelleting of cells by centrifugation and removal of the supernatant by aspiration, the cells were resuspended in the microcentrifuge tube in 1 mL of the Triton X-100 exposure medium prepared in spring water. The cells were exposed for 2 h, after which they were spun down, and the cells were assayed for viability as described below.

2.4. Cell viability assays

2.4.1. Cell lines

Step-by-step protocols on how to perform three of the cell viability assays on animal cell lines are described in Ganassin et al. (2000) for the use of AB (Medicorp. Science, Inc., Montreal, PQ, Canada) and CFDA-AM (Molecular Probes, Eugene, OR, USA) and in Dayeh et al. (2003) for NR (3-amino-7-dimethylamino-2-methylphenanzine hydrochloride; Sigma Chemical Co., St. Louis, MO, USA). For the PI (Molecular Probes) assay, the medium with Triton X-100 was removed and replaced with 100 μL of PI (10 μg/mL) in L-15/ex per well. The microwell plates were incubated for 1 h at room temperature for the fish cells and at 37°C for the mammalian cells, after which fluorescence was measured. For all dyes, fluorescence as fluorescent units (FUs) was quantified with the CytoFluor Series 4000 microplate reader (Applied Biosystems, Foster City, CA, USA) at the respective excitation and emission wavelengths of 530 and 595 nm for AB, 485 and 530 nm for CFDA-AM, 530 and 645 nm for NR, and 530 and 620 nm for PI.

2.4.2. T. thermophila

After the 2-h exposure of *T. thermophila* to Triton X-100 had been terminated by centrifugation and aspiration, the cells were resuspended in working solutions of the fluorescent indicator dyes, which were prepared as for the cell lines. Ciliates were incubated at room temperature in the microcentrifuge tubes. Incubations were for 30 min in a combined AB and CFDA-AM solution and for 60 min in PI and in NR. Afterward a 100-μL aliquot from each tube was transferred into microculture wells of a 96-well plate in six replicates, and fluorescence was quantified using the CytoFluor microwell plate reader as described above.

2.5. Data analysis

For all assays except PI, the raw FUs for wells without cells but with the varying Triton X-100 concentrations (no-cell controls) were subtracted from the FUs for wells with both cells and the varying Triton X-100 concentrations. As the FUs for no-cell controls did not change with increasing Triton X-100 concentrations, this step was unnecessary in this study but when industrial effluents were evaluated, this was done because sometimes FUs in CFDA-AM and AB assays increased with increasing effluent concentration (Dayeh et al., 2002). The resulting values were expressed as a percentage of the FUs in wells with cells but no Triton X-100 (no-treatment controls). These values were averaged for each treatment concentration and standard deviations were calculated. For the AB, CFDA-AM, and NR assays, toxicity is indicated by a decrease in

values relative to the no-treatment control. For the PI assay, toxicity is revealed by an increase in FUs relative to the no-treatment control. As in the other assays, the FUs for no-cell controls in the PI assay were constant despite varying Triton X-100 concentrations, but in the PI assay these FUs were not subtracted from the FUs for wells with cells because the differences between the no-cell controls and the no-treatment controls were small, leading to more variable results when the treatment FUs were expressed as a percentage of the no-treatment control. All results were plotted using SigmaPlot (Jandel Scientific), and EC_{50} values were determined using the logistic function option in SigmaPlot for dose–response curves (Ganassin et al., 2000). EC_{50} values for the different assays were compared by a one-way analysis of variance. For p values less than 0.05, the data were further examined by the Tukey–Kramer multiple comparisons test.

3. Results

For the animal cell lines, all four fluorescent dyes indicated a loss of viability in cultures exposed to increasing concentrations of Triton X-100, but some dyes appeared better than others in detecting surfactant cytotoxicity. NR appeared to be the most sensitive (Figs. 1–3), giving the lowest EC_{50}'s and revealing no differences between the EC_{50}'s for the five cell lines (Table 1). AB detected cytotoxicity at lower Triton X-100 doses in cultures of piscine cells (Fig. 1) than in mammalian cells (Figs. 2 and 3), whereas the PI assay indicated that the piscine cells were less susceptible (Table 1). CFDA-AM was the least sensitive dye, yielding the most variable results within a cell line and between cell lines. Sometimes with the human cell lines the fluorescence readings in the CFDA-AM assay declined with increasing Triton X-100 but did not reach 50% of the control (Fig. 3; Table 1).

For *T. thermophila*, AB, NR, and PI indicated an increasing loss of viability upon exposure to increasing concentrations of Triton X-100, but CFDA-AM did not (Fig. 4). Both AB and NR gave similar results, whereas the PI assay was more variable (Table 1). In contrast to the AB and NR assays, fluorescence readings in the CFDA-AM assay unexpectedly increased relative to the control. When cultures treated with high Triton X-100 concentrations were observed under the phase contrast microscope, cell debris was evident, indicating that the cells had indeed been lysed.

T. thermophila was not as susceptible as the animal cell lines to Triton X-100 (Fig. 4). For the AB and NR assays, the EC_{50}'s with *T. thermophila* were significantly higher than the combined EC_{50}'s for all the animal cell lines. When the EC_{50}'s for least and most sensitive animal cell lines were used as a point of comparison,

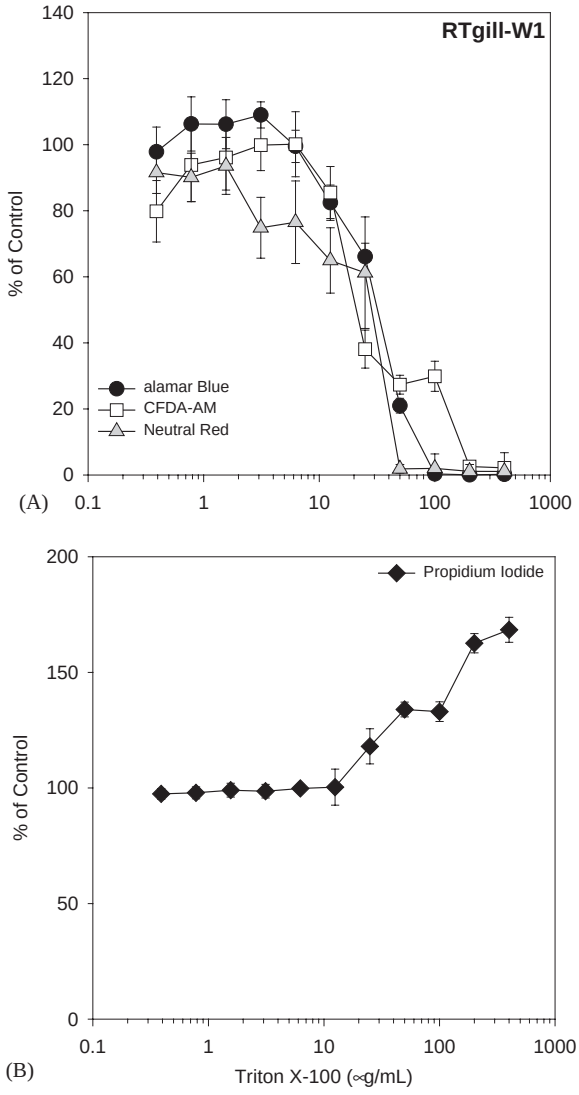

Fig. 1. Viability of rainbow trout RTgill-W1 cells upon exposure to Triton X-100 for 2 h in L-15/ex. Cell viability was measured with fluorescent indicator dyes: AB, CFDA-AM, NR (A), and PI (B). Results are expressed as a percentage of the readings in control wells exposed to L-15/ex alone. The data points represent the means of five culture wells with standard deviations.

they were approximately 2- to 6.5-fold higher with *T. thermophila* than with the animal cell lines (Table 1). However, the EC_{50}'s with PI were similar between the animal cell lines and *T. thermophila*.

4. Discussion

The cell viability assays with the fluorometric dyes AB, NR, and PI appeared suitable for evaluating the cytotoxicity of Triton X-100 to mammalian and piscine cells in culture. The EC_{50}'s, which ranged between 18 and 70 μg/mL for the human cells (Caco2 and HepG2), were broadly similar to the values obtained by others

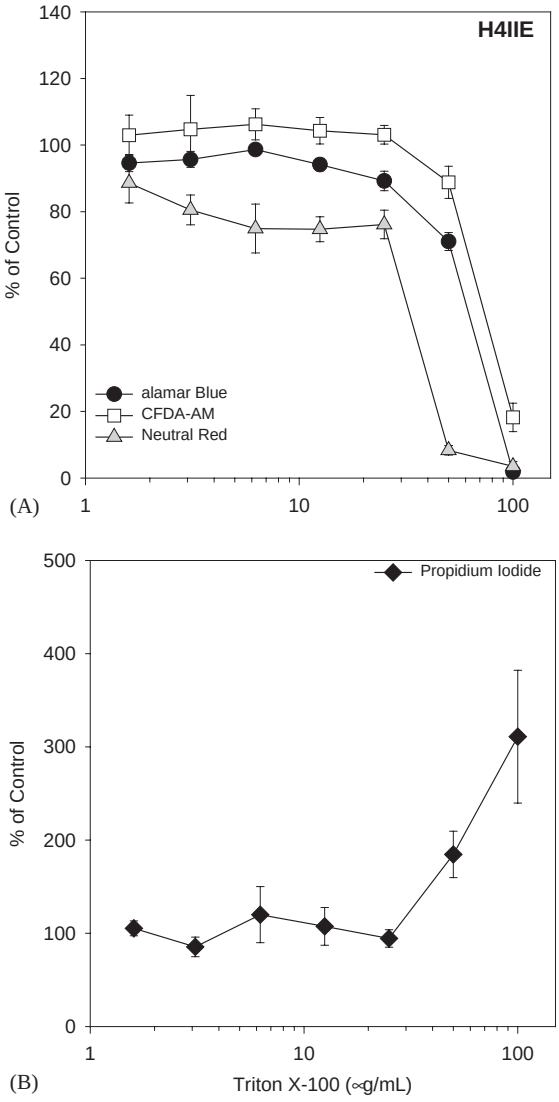

Fig. 2. Viability of rat H4IIE cells upon exposure to Triton X-100 for 2 h in L-15/ex. Cell viability was measured with fluorescent indicator dyes: AB, CFDA-AM, NR (A), and PI (B). Results are expressed as a percentage of the readings in control wells exposed to L-15/ex alone. The data points represent the means of five culture wells with standard deviations.

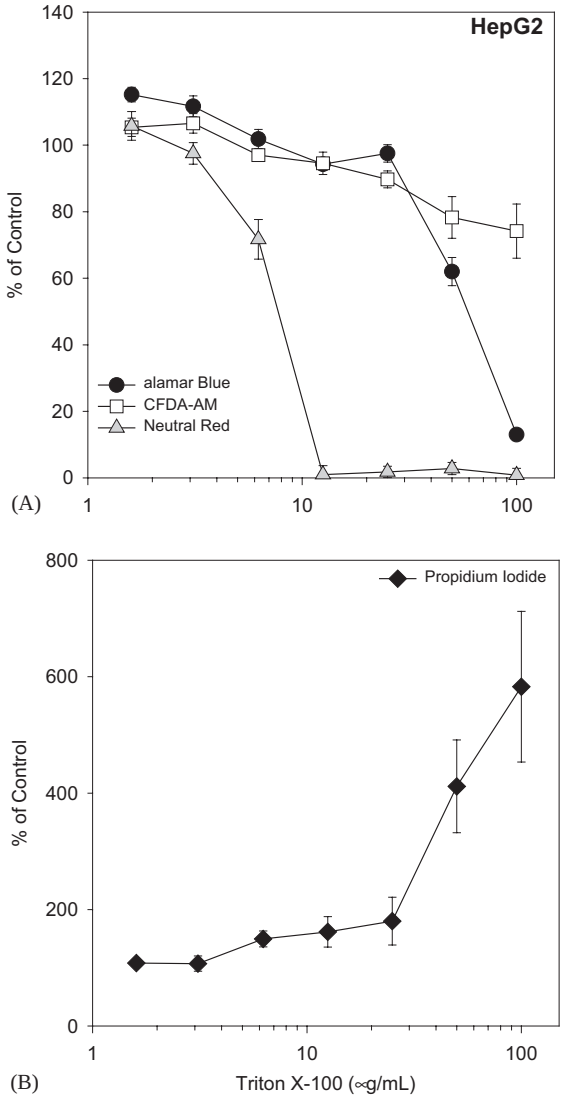

Fig. 3. Viability of human HepG2 cells upon exposure to Triton X-100 for 2 h in L-15/ex. Cell viability was measured with fluorescent indicator dyes: AB, CFDA-AM, NR (A), and PI (B). Results are expressed as a percentage of the readings in control wells exposed to L-15/ex alone. The data points represent the means of five culture wells with standard deviations.

upon exposing mammalian cell cultures to Triton X-100 for 1–3 h but using different indicators of cytotoxicity. Triton X-100 EC_{50} values ranged between 41 and 85 μg/mL for human fibroblasts (Arechabala et al., 1999), and for the human monocyte/macrophage cell line U937 (Jelinek and Klocking, 1998), with respectively MTT and sodium 3,3′-{1-[(phenylamino)carbonyl]-3,4-tetrazolium}-bis(4-methoxy-6-nitro)benzene sulfonic acid hydrate as the endpoints, and on primary rabbit corneal epithelial cells, with MTT and lactate dehydrogenase release as endpoints (Grant et al., 1992). EC_{50} values for Triton X-100 cytotoxicity to human fibroblasts were reported as 34 μg/mL using NR (Arechabala et al., 1999) and 97 and 100 μg/mL, respectively, with NR and AB (Lee et al., 2000). The tendency toward lower EC_{50} values for the current study might have been due to exposures in a very simple medium, L-15/ex; exposures in the other studies were done in complex basal media. The simplicity of L-15/ex may allow the expression of cytotoxicity at lower doses, as fewer potentially protective compounds would be present. The fish cell lines were as susceptible to Triton X-100 as the mammalian cell lines as judged by the EC_{50}'s for the assays using AB, NR, and PI.

CFDA-AM was problematic as a viability dye for studying Triton X-100. CFDA-AM indicates cytotoxicity through the loss from cells of esterases that are responsible for converting CFDA-AM to the fluorescent

Table 1
Summary of EC_{50} values for cell viability assays on cells of fish, mammals, and the protozoan *T. thermophila* exposed to Triton X-100 for 2 h

Organism	Cell line	Triton X-100 EC_{50} (μg/mL)											
		Alamar Blue			CFDA-AM			Neutral red			Propidium iodide		
		Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*	Mean	SD	*N*
Rainbow trout (*O. mykiss*)	RTgill-W1	41	11	4	79	56	3	32	6	3	75	10	4
	RTL-W1	26	4	3	63	31	3	19	10	2	77	6	2
Human (*Homo sapiens*)	Caco2	56	2	2	124	16	2	18	5	2	49	14	2
	HepG2	70	17	2	N/A[a]	N/A[a]	2	23	22	2	39	2	2
Rat (*Rattus norvegicus*)	H4IIE	59	—	1	71	—	1	26	—	1	52	—	1
T. thermophila	B1975	128	6	2	N/A[a]	N/A[a]	2	114	10	2	79	33	2

[a] N/A indicates that dose–response curves showing a 50% decline in cell viability were not obtained.

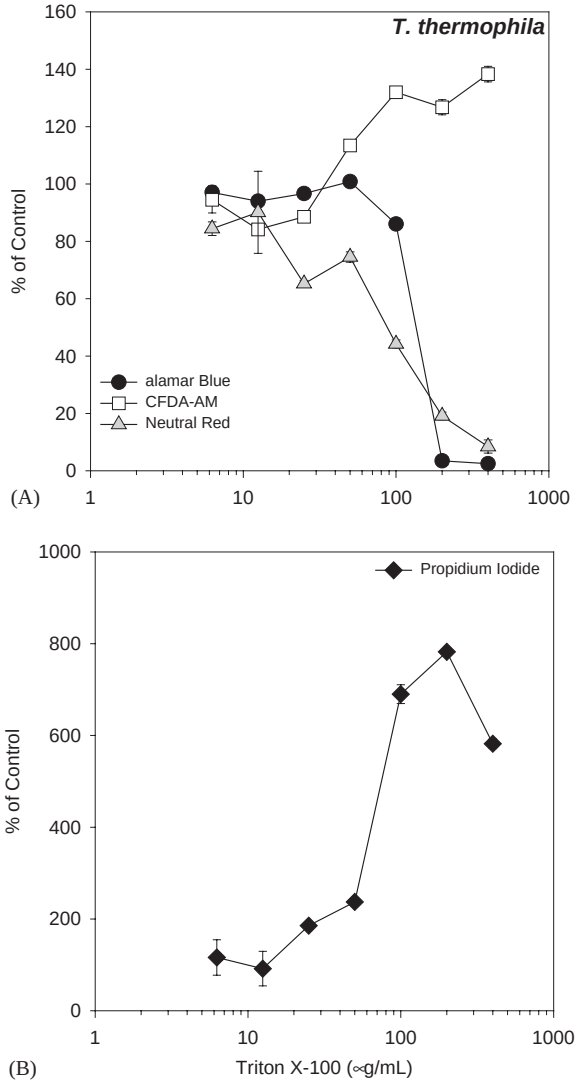

Fig. 4. Viability of the ciliate *T. thermophila* upon exposure to Triton X-100 for 2 h in spring water. Cell viability was measured with fluorescent indicator dyes: AB, CFDA-AM, NR (A), and PI (B). Results are expressed as a percentage of the readings in control wells exposed to spring water alone. The data points represent the means of five culture wells with standard deviations.

product, CF. The esterases can be lost when the cells are completely disrupted after termination of the toxicant exposure such that the cells do not remain attached to the microwell plates. In this scenario, the CFDA-AM results would be expected to mirror other measures of cell viability. With less complete cellular disruption, the esterases could be lost by leaking out of the cells during the toxicant treatment and then be removed when the toxicant-containing medium is discarded. If this leakage of esterases were incomplete, this measure of cytotoxicity would not be as severe as other measures. This appears to have occurred on occasion with the animal cell lines after surfactant treatment, especially with HepG2. In support of this scenario, Triton X-100 was observed to solubilize protein from rainbow trout gill epithelial cells more slowly than other surfactants (Partearroyo et al., 1991). Despite this insensitivity, CFDA-AM probably should be retained in a suite of cell viability assays to screen industrial effluent, as the dye works well with other classes of toxicants (Schirmer et al., 1998).

Three of the four fluorometric viability assays were used successfully to monitor viability in *T. thermophila* cultures after Triton X-100 treatment. AB gave consistent dose–response curves and, along with PI, was much easier to use than NR, which required the fixing of the cells and extraction of the dye. The results with these assays compared favorably with the results of studies on *T. pyriformis*, in which different exposure conditions and cytotoxic endpoints were used. *T. pyriformis* was exposed to Triton X-100 in growth medium (PPY) for 48 h and viability monitored as a loss of motility (Nicolau et al., 1999) or a decline in MTT reduction (Dias et al., 1999). Nearly all cells were dead at concentrations above 50 μg/mL in the study with *T. pyriformis* and above 200 μg/mL in the current study. The much shorter exposure period (2 h) in the current study likely accounts for the higher value. The expression of toxicity within 2 h likely happened because exposures were done in spring water, which, in contrast

to growth medium, would be expected to have few compounds that could interfere with the actions of toxicants.

In contrast to the other dyes, the CFDA-AM assay showed a slight increase rather than a decline in fluorescence values for *T. thermophila* exposed to increasing Triton X-100 concentrations. An explanation for this can be advanced by considering events likely occurring after cells had been pelleted to terminate Triton X-100 exposure and had been resuspended in the CFDA-AM solution to initiate the assay. For completely disrupted cells, esterases might be present in cell fragments, which could be pelleted and resuspended, and be more accessible to CFDA-AM than esterases in intact *T. thermophila*. For less damaged cells, the CFDA-AM might have more easily penetrated the surfactant-treated cells and reached intracellular esterases, or alternatively, esterases could have leaked out into the surrounding solution and more effectively reached the CFDA-AM substrate. These scenarios would account for the increased conversion of CFDA-AM to fluorescent CF.

Whether the relative toxicity and toxic mechanism of surfactants differ in the wide range of organisms potentially exposed to them is largely unknown. The use of multiple cytotoxic endpoints has the advantage of giving possible insight into the toxic mechanisms, as well as ensuring that in screens of potential toxicants a negative response is not missed. The results with the animal cell lines suggest that lysosomal activity, measured as the uptake and retention of NR, is more susceptible to Triton X-100 than energy metabolism, as measured with AB, or plasma membrane integrity, as measured with PI and CFDA-AM. More research should reveal whether the NR assay is as sensitive to other classes of environmental contaminants with different modes of action. However, the underlying cause of even the NR results might be general membrane damage. A common mechanism among organisms would make alternative test organisms attractive as predictors of toxicity. Even without complete knowledge of such a mechanism, some recent results are promising (Sandbacka et al., 2000). They compared the toxicity of surfactants, but not Triton X-100, to freshly isolated rainbow trout gill epithelial cells, *Daphnia*, and fish and found that the results with epithelial cells and *Daphnia* could be used to predict acute toxicity of surfactants to fish.

The results and procedures of the current study suggest that *Tetrahymena* could be helpful for screening industrial effluent for acute toxicity to fish and perhaps serve as a complement and/or substitute for fish and fish cells in toxicity testing. Using *T. thermophila* to screen for toxicants is advantageous relative to whole fish by reducing the amount of sample needed for testing, which reduces shipping costs, and time required to complete testing, which can aid decision-making. The susceptibility of the protozoan and animal cell lines to Triton X-100 appeared sufficiently close to make *Tetrahymena* a useful indicator of toxicity, although *T. thermophila* was approximately two-fold less sensitive as judged by AB and NR. Ciliates like *Tetrahymena* have a complex pellicle (Lynn and Corliss, 1991) and this likely makes them more resistant to lysis by surfactants. Although the EC_{50}'s for Triton X-100 are much higher than general environmental contamination levels (Partearroyo et al., 1991), the screening protocols are meant to detect episodic events, resulting in the release of high surfactant levels in industrial effluent. The LC_{50} of Triton X-100 adult zebrafish (*Danio rerio*) after 96 h of exposure was reported as 13 mg/L (Kovriznych and Urbancikova, 2001), which is lower than the EC_{50} values (Table 1) for *T. thermophila* of this study. However, the values are within the same order of magnitude and higher values with the protozoan are not unexpected given the much shorter exposure period. Interestingly, Seward et al. (2002) have shown that there is a good overall relationship between *T. pyriformis* and *Poecilia reticulata* toxicity to a variety of toxicants.

Whether *T. thermophila* and fish cells will show similar susceptibility to other classes of environmental toxicants remains to be seen. However, the fact that common cell viability assays can be used should make comparisons easier. The procedures of this study are rapid, which is an essential requirement for screening protocols. The speed is due to the ease with which the fluorometric viability assays can be performed but, as well, to the quickness of the cytotoxic response, which likely was enhanced by toxicant exposure being done in spring water. Being able to expose *T. thermophila* in spring water means that exposure to industrial effluent could be done directly, without additional processing. This will also speed up testing and reduce costs.

Acknowledgment

This research was supported by the Center for Research in Earth & Space Technologies (CRESTech).

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