FISH CRITICAL CELLULAR RESIDUES FOR SURFACTANTS AND
SURFACTANT MIXTURES

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Abstract—The feasibility of using in vitro methods to predict in vivo critical body residue (CBR) for single surfactants and mixtures by measuring the critical cell residue (CCR) in a hepatic fish cell line (PLHC-1C) was investigated. The CCR values were determined using radiochemical methods to measure the test compound partitioning between media and cells at varying concentrations for three distinctly different surfactants (anionic, cationic, and nonionic) and their mixture. The cell median effective concentration (EC50) values for hexadecyltrimethylammonium chloride ($C_{16}$TMAC), dodecyl hexaethoxylate ($C_{12}$E6), and sodium dodecylbenzene sulfonate ($C_{12}$LAS) ranged from 2.9 to 163.3 μM, a 54-fold difference. These cell EC50 values indicate that the cells are several-fold less sensitive to surfactants than whole organisms are. However, based on cellular residue levels for each surfactant and their mixture, only an approximately threefold difference was observed with a range of 0.6 to 1.8 mmol/kg. These concentrations correspond closely to in vivo body burdens (0.2–8 mmol/kg) associated with nonpolar organic or narcosis-acting compounds and their mixtures. The CCRs could provide an alternative and rapid technique to predict CBRs.

Keywords—Critical body residue Mixtures Surfactants In vitro

INTRODUCTION

Typically, aquatic toxicity is expressed as the median effective concentration (EC50) or median lethal concentration (LC50)—that is, as the concentration of toxicant (mg/L) in the media that results in a 50% effect (e.g., cell growth inhibition) or lethality, respectively. Aquatic toxicity also can be expressed as a dose (e.g., median lethal dose or median effective dose [ED50], body burden in μmol/kg) that is the concentration of toxicant measured in the organism. The critical body residue (CBR) theory infers that despite diverse LC50s, acutely toxic effects (mortality) occur at body burdens of 0.2 to 8 mmol toxicant/kg body weight, particularly for nonpolar organic or narcosis-acting compounds [1]. Importantly, the CBR has been shown to be essential in understanding mixture toxicity. That is, acute effects are found at body burdens from the sum of all the narcosis-acting compounds.

Surface-active agents (surfactants) are sorptive materials that readily partition to surfaces. They are used in laundry detergents, cleaning products, pharmaceuticals, and other applications. Surfactants typically have a polar attribute and a nonpolar hydrophobic component that leads to physicochemical properties and toxicity (structure–toxicity relationships) that support their inclusion as narcosis-acting compounds [2,3]. A recent study by Versteeg and Rawlings [4] verified this hypothesis with the anionic sodium surfactant dodecylbenzene sulfonate ($C_{12}$LAS), showing median lethal body burdens in various fish species (fathead minnow and channel catfish) and an amphipod (Hyalella azteca) to range from 0.21 to 0.59 mmol/kg.

In-house in vitro studies using a hepatic fish cell line (PLHC-1C) have shown that surfactants with diverse structures (anionic, nonionic, and cationic) have widely varying concentration–response curves and different EC50 values (unpublished data). Yet, when these surfactants were applied as mixtures to fish cells, they typically were found to be additive to partially additive, indicating that they have the same mode of action. Conclusive evidence, however, requires an understanding of the dose response of surfactants, especially in cellular systems. In addition, a need exists to relate toxicity as a function of body burden (i.e., CBR), especially as mixtures.

The cost of obtaining CBRs for whole organisms is high, because long exposures, depuration periods, and use of animals are required. An in vitro approach to obtain CBRs would lead to mechanistically supportable effects assessments. Also, this approach would provide evidence and criteria for inclusion of materials in mixture-toxicity assessments. Animal usage would be reduced as well. Importantly, these three factors could yield lower cost and faster, yet reliable, results that could be used in environmental risk assessments. Hence, in vitro techniques to measure a critical cell residue (CCR), which is analogous to the CBR, could provide an alternative and rapid approach to predict CBRs.

The objectives of the present study were to assess the applicability of measuring CCR in a hepatic fish cell line (PLHC-1C) and to determine the feasibility of using in vitro methods to predict in vivo CBRs for single surfactants and mixtures. Concentration (media-based) and cellular dose–response relationships for three structurally diverse surfactants, both as single components and as mixtures, to PLHC-1C cells were determined. The CCRs were determined for three surfactants—hexadecyltrimethylammonium chloride ($C_{16}$TMAC), dodecyl hexaethoxylate ($C_{12}$E6), and $C_{12}$LAS—and for mixtures containing all three surfactants.

MATERIALS AND METHODS

Test chemicals

Nonradio-labeled $C_{12}$E6 was obtained from both Nikko Chemical (Tokyo, Japan) and Fluka (Milwaukee, WI, USA); only trace impurities were present. The $^{14}$C$_{12}$E6 isotope (purity,
trimethylammonium chloride (C16 TMAC).

97% pure. Radiochemical 14 C 16 TMAC was synthesized by The Costar at-bottom polystyrene plates, and Corning from Sigma Chemical (St. Louis, MO, USA). Microplates were obtained from TCI America (Portland, OR, USA), with a purity of 96.2%. The 14 C 12 LAS was synthesized by The Procter & Gamble Company (Cincinnati, OH, USA). An imidazoline-ethoxylate (C12 E6), (C16 TMAC), (Bio-Rad Laboratories, Hercules, CA, USA). The TLC plates were Whatman LK5 Silica gel 150 A, 250 micron (MDX, Brentford, UK). Microplate absorbances were read on a Microplate Spectrophotometer (Molecular Devices Biolog Reader, SpectraMAX 250; Sunnyvale, CA, USA). Liquid scintillation counting of radiochemicals was performed in Ultima Gold Scintillation Cocktail (Packard, Wellesley, MA, USA) on a Beckman LS 6000 counter (Fullerton, CA, USA). The TLC plates were scanned on a System 200 Imaging Scanner and then analyzed using Win-Scan Radio TLC Software (Ver 2.2(5); Bioscan, Washington, DC, USA).

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PLHC-1C cell line and culture methods

The PLHC-1C cells were used in the present study for several reasons. First, they can be grown at very high densities in culture, which provides a large range of signal to noise ratios in cytotoxicity tests. Second, they have maintained some liver functions, including inducible P4501A activity [5]. Hence, measured cytotoxicity may incorporate the action of toxic metabolites. We also have used these cells to investigate biotransformation rates of surfactants [6] (http://www.erasm.org/study.htm). The PLHC-1C cells were derived from a hepatocellular carcinoma that was induced in Poeciliopsis lucida, a desert topminnow, using 7,12-dimethyl-benz[a]anthracene [7]. Cells were obtained from L. Hightower (University of Connecticut, Storrs, CT, USA) and cryogenically preserved in liquid nitrogen. Cell cultures were grown and tested in 1-glutamine (4 mM)– and fetal bovine serum (5%)–supplemented, CO2-independent media. Typically, the cultures were used within 18 passages of being removed from cryogenic storage. Cells were grown to confluency in T25 flasks and then subcultured by dissociating with trypsin (0.05%)–ethylenediaminetetra-acetic acid, tetrasodium salt (EDTA; 0.53 mM). Cell cultures of PLHC-1C cells have a population-doubling time of approximately 35 to 40 h (at 30°C) and form a dense, columnar monolayer when confluent. All culture media and reagents were obtained from Invitrogen, Life Technologies (Carlsbad, CA, USA).

General approach

The objective was to determine the applicability of the CBR theory to a hepatic fish cell line (PLHC-1C) as a CCR and the feasibility of using in vitro methods to predict in vivo CBRS for single surfactants and mixtures. The objective was achieved by determining the dose–response relationships and test system partitioning of three structurally diverse surfactants as single components and mixtures to PLHC-1C cells (Fig. 1). The quantities of the surfactant per cellular weight were determined at the concentrations near the EC50. Cytotoxicity, partitioning and distribution data, and cellular weights were necessary to determine CCRs. Cytotoxicity was determined using neutral red uptake (NRU) and SRB assays. Surfactants associated with the cells were determined by measuring the radioactivity by liquid scintillation counting. For mixture experiments, the distribution of each surfactant in media and within the cells was determined via TLC. Cellular weights were estimated using NRU, SRB, and cell counts.

Cellular weight and relationship to total protein

Confluent cells in three T25 flasks were detached using 1.5 ml of trypsin-EDTA, combined and diluted with 9 ml of media. A 1:8 dilution of cells in Dulbecco’s phosphate-buffered saline was counted using a hemocytometer. Aliquots (1.5 ml) of the cell dilution were centrifuged in tared polyethylene 5-ml vials at 200 g using a microcentrifuge. The supernatant was examined microscopically, and cells were counted to determine the adequacy of centrifugation. The majority of supernatant was carefully removed using a Pasteur pipette and the remainder using the capillary action of a 20-μl micropipette. Cells and vials were then reweighed and the difference determined as the weight of the cells (expressed as mass/cell).

The relationship of cell numbers to total protein (via SRB) was determined using methods described by Skehah et al. [8]. A series of cell dilutions was seeded into two 96-well plates
and incubated for 24 h. In one plate, three to four wells of each cell concentration were counted with a hemocytometer. The other plate was assayed for SRB protein. The relationship of total protein to cell numbers (and, hence, to mass) was determined via linear regression.

**Cytotoxicity tests**

Microtiter plates were seeded with a density of $4 \times 10^5$ cells/cm$^2$ and incubated for approximately 24 h at 30°C before dosing. Dosing solutions were prepared on the day of exposure from test chemical stocks and media. Test chemical concentrations were spiked into wells at nominal concentrations ranging from no effect compared to total effect (e.g., mortality or lysis). Twenty-four hours after exposure, NRU and SRB assays were performed sequentially on the same microwells. For NRU, dosing media was removed from plates and replaced with 0.004% neutral red in media. Neutral red dye passively diffuses into the cell, where it is trapped by the acidic environment of the lysosomes of viable cells. After allowing uptake for 2 to 3 h, neutral red was then extracted from fixed cells with extraction solution (1% acetic acid and 50% ethanol) and read on a spectrophotometer at 630 nm [9].

Plates were air-dried before staining with 0.4% SRB in 1% acetic acid. Next, the plates were rinsed four times with 1% acetic acid. Plates were air-dried before staining with 0.4% SRB in 1% acetic acid. Next, the plates were rinsed four times with 1% acetic acid to remove excess SRB. Remaining stain was solubilized with a 10 mM Trizma base and the absorbance read at 540 nm. Responses were expressed as a percentage of the average control response. The concentration and dose–response curves and EC50/ED50 values, based on percentage control, were determined using either a four-parameter sigmoid $[y = a + b/(1 + \exp(-(x - c)/d))]$ or a log dose response $[y = a + b/(1 + (x/c)^d)]$ regression, where $a$ represents the lower plateau, $b$ represents the height of the transition, $c$ represents the midpoint of the transition, and $d$ represents the steepness of the transition. The regression used was based on the best fit of the two equations. Confidence limits (95%) were taken from prediction intervals defined by TableCurve 2D (Ver 4; Systat Software, Point Richmond, CA, USA).

**Partitioning or CCR studies**

Dosing solutions were comprised of both $^{14}$C-radiolabeled and unlabeled surfactants. Exposure details for the single chemical tests are summarized in Table 1. Two assays were conducted on C16MTAC to verify reproducibility.

Two 96-well microplates with PLHC-1C cells were dosed per surfactant. One plate was used for NRU and SRB assays; the other plate was used for surfactant partitioning and distribution analysis. In addition, a cell-free blank plate was used for background subtraction. All three plates were sampled after 24-h incubation.

For single-chemical exposures, concentration ranges were selected to closely bracket the EC50 values, with several levels corresponding to the steep slope of the concentration–response curve. Ten concentrations with six replicates per concentration were used per chemical. The concentration of radiochemical used depended on the results of preliminary partitioning experiments and specific activity of individual radiochemical. For example, less radioactivity was used for C16MTAC experiments than for C12LAS and C12E6 experiments because of the greater expected partitioning to cells. These data also are summarized in Table 1 for single chemicals.

**Mixture studies**

The basis used for assessing effects of mixtures was the toxic unit (TU). The TU is the concentration of a chemical in an organism divided by the concentration causing a special effect—in this case, the EC50. Experimental mixture concentrations included 0.6, 0.8, 0.9, 1.0, 1.1, and 1.3 TU. Therefore, the concentration of each toxicant ($i$) was calculated by multiplying the TU by the EC50 (TU = $i/EC50$). That is, mixture experiments were designed to closely bracket the EC50 value. The molar ratio of the surfactants in the mixture tests were equal to the ratio of their toxicity (EC50) values. Each surfactant stock was diluted in media before being mixed together, thereby minimizing surfactant–surfactant interactions. Mixture experiments required sufficient radiolabeled amounts of each surfactant to be detectable by TLC-RAD. Two mixture experiments were conducted (referred to as mixture experiments 1 and 2). The radioactivity of C16MTAC was increased in the second experiment to facilitate TLC and recovery. Exposure range and radioactivity used for the mixture experiments are summarized in Table 2.

After a 24-h exposure, in one seeded microplate the media were carefully collected using a pipette from three wells for each concentration, and the radioactivity was determined via liquid scintillation counting. The cells were collected from at least three control wells and selected other dosed wells for enumeration. These samples were counted manually using a

<p>| Table 1. Exposure details for single-surfactant critical cell residue experiments |
|---------------------------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Exposure range (µM)</th>
<th>% Radiolabeled surfactant</th>
<th>% Unlabeled surfactant</th>
<th>Approximate dpm range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dodecyl hexaethoxylate</td>
<td>7.1–113.6</td>
<td>7.1</td>
<td>92.9</td>
</tr>
<tr>
<td>Sodium dodecylbenzene sulfonate</td>
<td>60–200</td>
<td>0.6</td>
<td>99.4</td>
</tr>
<tr>
<td>Hexadecyltrimethylammonium chloride</td>
<td>0.4–6.4</td>
<td>14.2</td>
<td>85.8</td>
</tr>
</tbody>
</table>

*Two experiments were done.

- **Table 2. Exposure details for surfactant mixture experiments**

<table>
<thead>
<tr>
<th>Exposure range (µM)</th>
<th>% Radiolabeled surfactant</th>
<th>% Unlabeled surfactant</th>
<th>Approximate dpm range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dodecyl hexaethoxylate</td>
<td>10.1–21.0</td>
<td>81.0</td>
<td>19.0</td>
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<tr>
<td>Sodium dodecylbenzene sulfonate</td>
<td>32.6–67.9</td>
<td>5.7</td>
<td>94.3</td>
</tr>
<tr>
<td>Hexadecyltrimethylammonium chloride</td>
<td>0.96–2.0</td>
<td>51.1</td>
<td>49.9</td>
</tr>
</tbody>
</table>
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Fig. 2. General procedure outline for cell burden determination.

TLC – Thin Layer Chromatography; LSC – Liquid Scintillation Counting

Fig. 3. Radiograph thin-layer chromatograms of mixture surfactants, dodecyl hexaethoxylate (C\textsubscript{12}E\textsubscript{6}), dodecylbenzene sulfonate (C\textsubscript{12}LAS), and hexadecyltrimethylammonium chloride (C\textsubscript{16}TMAC) in (A) cells and (B) media. Experimental mixture concentration was 1.0 toxic unit.

The distribution of surfactants was determined by TLC. The TLC plates were eluted with solvent conditions consisting of chloroform (140 ml), methanol (80 ml), water (6 ml), and formic acid (2 ml). Plates were scanned for a maximum of 10,000 counts or 120 min on an imaging scanner. All scans were background subtracted. The percentage of each surfactant as determined by TLC in combination with the partitioning percentage was used to calculate the amount of each surfactant. An example TLC of a cell and media mixture sample is shown in Figure 3.

RESULTS

The PLHC-1C cells cultured in and harvested from culture flasks were used for weight determination. The mean cell count for determination of cell weight was $3.6 \times 10^6$ (SD $\pm 0.9 \times 10^6$) cells/mL. The average weight of cells in 1.5-ml samples ($n = 8$) was 0.02016 (SD $\pm 0.00421$) g. The calculated average cell weight was $3.73 \times 10^{-2}$ mg/cell. This measured cell weight was used for calculations of cell burdens assuming that cell weight was constant in controls and exposed cells. The cell weight is analogous to weights used in critical body burden calculations; hence, cell burdens are expressed as mmol/kg for comparative purposes.

The plot of absorbance of the SRB assay versus cell counts was linear in duplicate experiments, with coefficients of determination of 0.96 and 0.97. Single-point calibrations using SRB assay and manual cell counts from control wells ($n \geq 3$) were used to calculate sample cell counts. Sample SRB assay values (as a percentage of control) were multiplied by the control cell count average to determine the sample cell count. This count was then multiplied by the previously determined cell weight. It was necessary to count the control wells in each experiment because of variation in SRB sensitivity.

Median effective concentrations (media) and doses (cellular) for C\textsubscript{12}E\textsubscript{6}, C\textsubscript{12}LAS, and C\textsubscript{16}TMAC are presented in Table 3. The most toxic surfactant was C\textsubscript{16}TMAC, followed by C\textsubscript{12}E\textsubscript{6} and C\textsubscript{12}LAS, respectively. The lowest EC50 value was 2.9 $\mu$M (C\textsubscript{16}TMAC), and the greatest was 163.3 $\mu$M, a 55-fold range. The portion of radiolabel in the test systems associated with the media ranged from 75 to 100% for C\textsubscript{12}E\textsubscript{6}, 94 to 97% for C\textsubscript{12}LAS, and 39 to 56% for C\textsubscript{16}TMAC. Likewise, the radiolabel portion in the test systems associated with the cell ranged from

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>EC50 (M)</th>
<th>95% Confidence limits (M)</th>
<th>ED50 (mmol/kg)</th>
<th>95% Confidence limits (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dodecyl hexaethoxylate(^a)</td>
<td>49.3</td>
<td>45.3–53.2</td>
<td>1.3</td>
<td>1.0–1.6</td>
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<tr>
<td>Dodecylbenzene sulfonate</td>
<td>44.6</td>
<td>31.6–58.0</td>
<td>0.6</td>
<td>0.3–1.1</td>
</tr>
<tr>
<td>Hexadecyltrimethylammonium chloride</td>
<td>163.3</td>
<td>152.1–175.9</td>
<td>1.5</td>
<td>1.0–2.3</td>
</tr>
<tr>
<td></td>
<td>2.9</td>
<td>0.2–Not defined</td>
<td>0.6</td>
<td>0.1–1.5</td>
</tr>
</tbody>
</table>

\(^a\) Two experiments were done.
Table 4. Comparison of critical cell residue (CCR) to other toxicological data and physical/chemical properties

<table>
<thead>
<tr>
<th></th>
<th>EC50 a (µM)</th>
<th>CCR (mmol/kg)</th>
<th>CBR b (mmol/kg)</th>
<th>Log P (Kow) c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dodecyl hexaethoxylate d</td>
<td>49.3</td>
<td>1.3</td>
<td>NA a</td>
<td>4.8</td>
</tr>
<tr>
<td>Sodium dodecylbenzene sulfonate</td>
<td>44.6</td>
<td>0.6</td>
<td>NA a</td>
<td>0.6</td>
</tr>
<tr>
<td>Hexadecyltrimethylammonium chloride</td>
<td>163.3</td>
<td>1.5</td>
<td>0.3–1.4 f</td>
<td>3.3</td>
</tr>
</tbody>
</table>

EC50 = median effective concentration.
CBR = critical body residue.
Syracuse Research [24].
Two experiments were done.
NA = not available.
Versteeg and Rawling [4].

1 to 7% for C12E6, 1 to 7% for C12LAS, and 19 to 29% for C16TMAC. The unaccountable radiolabel material fraction ranged from 0 to 22% for C12E6, 1 to 5% for C12LAS, and 14 to 42% for C16TMAC. Compared to the media-based effect levels, a much narrower range was observed for each surfactant with respect to internal doses conforming to the median effect. The ED50 values ranged by a factor of 2.5, from 0.6 mmol/kg (C16TMAC) to 1.5 mmol/kg (C12LAS). Results from this individual compound experiment along with the whole-organism CBR for C12LAS [4] and calculated Kow values are summarized in Table 4.

Two mixture experiments were conducted. Test mixtures contained varying concentrations of equal TUs of each compound. Neutral red uptake was used for assessing cytotoxicity in mixture experiment 1, and SRB was used in mixture experiment 2 (SRB results for mixture experiment 1 were not usable because of unknown interferences). The external concentrations of each surfactant at the EC50 for mixture 1 and mixture 2 were, respectively, 17.4 and 19.6 µM for C12E6, 57.6 and 64.8 µM for C12LAS, and 1.0 and 1.2 µM for C16TMAC. The EC50 is the nominal external concentration at which a 50% decrease from the control cell density is observed. The EC50 for mixture 1 and mixture 2 corresponded to 1.1 and 1.2 TU, respectively, indicating additive toxicity.

The internal cell dose (CCR or ED50, with lower and upper confidence limits) for all compounds at the EC50 of mixture 1 and mixture 2 was 1.8 mmol/kg (1.6–2.0) and 0.7 mmol/kg (0.6–undefined), respectively. The distributions of surfactants dosed into the test system as well as the distributions of surfactants found in the cells are depicted graphically in Figure 4.

For both experiments, at least 90% of the radiolabel was associated with the media. The portion of radiochemicals associated with the cell ranged from 4 to 6%. The unaccountable fraction in the mixture test system ranged from 2 to 12%. This fraction refers to the difference in the spiked amounts in cells or media and that which is associated with well walls and/or nonextractable test materials. Of the radiolabel found in the cells during the first experiment, 14C12 LAS was found in the highest concentration, followed by 14C12E6 and 14C16TMAC. (The second experiment dosed 14C16TMAC at a greater level; hence, more was observed, contributing to the proportion found in cells.) Approximately an equal distribution of the three surfactants was observed throughout the TU range dosed into the test system.

![Fig. 4. Distribution in mixture experiments. The distribution of total radioactivity in the test system among the media, cells, and other is depicted for mixture experiment 1 (A) and mixture experiment 2 (B). The distribution of each test chemical associated with the cells comprising cell burden is depicted for mixture experiment 1 (C) and mixture experiment 2 (D). C12E6 = dodecyl hexaethoxylate; C12LAS = sodium dodecylbenzene sulfonate; C16TMAC = hexadecyltrimethylammonium chloride.](image)
DISCUSSION

The ED50 for each experiment was determined from the percentage control versus total cell burden (mmol/kg). Figure 5 provides cytotoxicity as percentage control versus mixture TUs, total cell burden versus TUs, and percentage control versus total cell burden for each experiment. The total cell burden associated with TUs dosed into the system reveals a monotonic increase to the highest concentration tested (1.25 TU). Cell burdens associated with the ED50 in the mixture experiments ranged from 0.7 to 1.8 mmol/kg.

Based on extracellular concentrations (i.e., media), in vitro cytotoxicity assays in the present study showed that C16TMAC was the most toxic surfactant, followed by C12E6 and C12LAS, respectively (EC50, 2.9, 46.7, and 163.3 μM, respectively). A comparison of the EC50 values for C12LAS between PLHC-1C cells and several aquatic organisms (amphipod H. azteca, the bivalve Corbicula fluminea, fathead minnow, and bluegill sunfish [4]) indicated that the in vitro system was more than 100-fold less sensitive. Similar findings were reported by Sandbacka et al. [10], who compared the acute toxicity of cationic and zwitterionic surfactants to fish cells (hepatocytes and gill epithelia from rainbow trout), Daphnia magna, and rainbow trout in vivo. For both surfactant classes, in vitro systems were several-fold less sensitive (greater EC50 values) than organisms were. Several other authors [11–14] also have reported that in
Ed50 values) for single surfactants in PLHC-1C cells ranged 1.6 mmol/kg wet weight, corresponding to the lower end range. Even so, why the major discrepancy between in vitro and in vivo effect levels? The present study has provided another avenue of effect comparisons based on internal concentrations or critical cell and body residues.

The relationship of body residue concentrations with lethality (EC50) was proposed by McCarty et al. [1] as the CBR. The CBR for narcotics corresponded to approximately 2.5 mmol/kg lipid [18]. Assuming an average lipid content of 5%, the CBR also was expressed as 50 mmol/kg lipid. Similarly, Van Wezel and Opperhuizen [19] found molar concentrations associated with death for narcotic-acting chemicals ranged from 2 to 8 mmol/kg organism or 40 to 160 mmol/kg lipid. DiToro et al. [20] reported body burdens associated with chronic endpoints for narcotic chemicals, which ranged from 2.16 to 6.94 mmol/kg lipid, whereas Verhaar et al. [21] indicated a range of 0.2 to 0.8 mmol/kg for a chronic effects threshold, a factor approximately 10-fold lower than what was found for acute toxicity effects. Versteeg and Rawlings [4] reported that mean lethal body burdens for C12LAS surfactant toxicity [4]. Furthermore, when expressed as TUs, the mixtures were found to be additive, suggesting a similar mode of action (e.g., narcosis).

The present study supports further evaluation of in vitro systems as surrogates for whole organism–based toxicity assays, particularly for prediction of tissue dose or CBR. The key advantages of assessing toxicity using tissue concentrations instead of administered doses are the minimization of issues associated with bioavailability and analytical shortcomings of poorly soluble materials, which can pose difficulties for mixture assessments [22]. Alternatively, understanding bioavailable concentrations in the in vitro system’s exposure media might allow a better comparison to external concentration effect levels from in vivo tests (e.g., LC50). For example, Heringa et al. [23] demonstrated that serum albumin at 5%, an amount commonly used in cell-based assays, bound 80% of tritiated estradiol. As a consequence, Heringa and coworkers concluded that use of nominal (or total measured values that do not discriminate bound from unbound material) concentrations, as often are employed during in vitro tests, can obscure quantitative determinations. We agree, because most in vitro tests use nominal concentrations in media; thus, the expressed toxicity often is not consistent with toxic thresholds observed with organisms.

REFERENCES


