Development of a Triton X-100 replacement for effective virus inactivation in biotechnology processes

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After the transmission of human viruses through plasma derivatives had been recognized in the early 1980s, virus inactivation and then removal steps have been implemented into their manufacturing processes. These measures have kept plasma derivatives safe ever since and have also served as a barrier against more recently emerging viruses. Given the success of these interventions, they have also been embedded into the manufacturing processes for cell-derived biological medicinal products. The most effective inactivation process for lipid-enveloped viruses is treatment by detergents or combinations of solvents and detergents, and thus, these processes have been almost universally adopted. One of the most widely used detergents, Triton X-100, has recently raised environmental concerns because one of its degradation products possesses hormone-like (estrogen-mimetic) activity that may act on wildlife. Consequently, use of the chemical in the European Union will ultimately be prohibited. The current study was conducted to establish an environmentally friendly detergent alternative to Triton X-100 with fully equivalent efficacy in biotechnological use. A newly synthesized compound, named Nereid, as well as Triton X-100 reduced, seem to satisfy these requirements, and thus may be suitable replacements for Triton X-100.

KEYWORDS
detergent, SD treatment, Triton X-100, virus clearance, virus inactivation

1 | INTRODUCTION

In response to the transmission of viruses through plasma derivatives in the early 1980s, the solvent-detergent (S/D) treatment was developed to inactivate lipid-enveloped viruses, and this technology is the most effective and robust method used for this purpose to date.

Over the three decades of use, mixtures of the solvent tri-n-butyl phosphate (TNBP) with detergents such as Triton X-100 (TX-100) and/or Polysorbate 80 (PS80, Tween 80) are widely considered most effective and have thus found wide
application in the medical plasma derivatives as well as biotechnology industry. It is interesting to note that the exact origins of the specific combinations are not entirely clear and may even have been somewhat fortuitous.\(^1\)

The recent observation that degradation products of TX-100 may have detrimental effects on the environment\(^3\) will result in restrictions on its use by the European Chemicals Agency.\(^4,5\) Thereby, the affected pharmaceutical industry, among others who use far larger quantities, now face the challenge of finding an alternative detergent to eventually replace TX-100.

Beyond being environmentally friendly, the ideal replacement candidate first needs to serve its original purpose, and thus have equivalent virus inactivation capabilities for all established process conditions, including at ambient as well as at cold temperatures. Furthermore, the alternative should have similar properties as TX-100 for manufacturing, that is, no negative influence on the often rather delicate biomolecules of interest, good water solubility with low propensity to foaming, easy detection by sensitive, simple and easily validated analytics, and effective removal by subsequent purification process steps. Finally, it would have to feature a favorable pharmacological toxicology as well as biodegradability profile. If indeed a candidate with these characteristics could be identified, the replacement of TX-100 would represent a minimal manufacturing process change only, and thus, the corresponding regulatory submissions might be limited to the Chemistry, Manufacturing, and Controls Section, rather than a demanding preclinical or even clinical evaluation.

Following the desire for minimal differences between TX-100 and the potential replacement candidates as a design principle, the new molecule should ideally be a nonionic and polyethylene glycol (PEG)-based detergent with a low critical micelle concentration (CMC). Different from TX-100, however, it should not contain the phenol entity, as this chemical group is revealed when the molecule is metabolized in the environment to become an estrogen receptor-binding pseudohormone.\(^3,6\) Alternatively, ready biodegradability of the new molecule or its degradation to nontoxic metabolites would be desirable features.

An investigation comparing four potential replacement candidates to the traditionally used TX-100 (Table 1) is presented here.

The original use of the two-component S/D treatments was somewhat fortuitous, and preliminary evidence had suggested that even the use of a single detergent could result in dependable virus inactivation. It was, therefore, tempting to evaluate whether now that the manufacturing processes for highly regulated medicinal products needed change anyway, the replacement of the traditional S/D mixtures by a single detergent was feasible. If indeed the use of a single detergent could result in similarly robust and effective virus inactivation, the respective process segment and its analytical monitoring could become simpler during biopharmaceutical manufacturing operations, and the effectiveness gain could help to offset some of the substantial efforts necessary as part of the change in process.

## MATERIALS AND METHODS

### 2.1 Detergents

The following commercially available chemicals for S/D treatment were used as received from the vendors: Triton X-100 (Merck, Cat. no. 108643), Triton X-100 reduced (Sigma Aldrich, Cat. no. X100RS), Brij C10 (Sigma Aldrich, Cat. no. 388858), Polysorbate 80 (PS80; Merck, Cat. no. 817061), TNBP (Merck, Cat. no. 100002), and Polysorbate 20 (PS20; Sigma Aldrich, Cat. no. 44112). Nereid is a proprietary compound synthesized by Takeda's R&D group (Vienna, Austria) for which a patent application has been filed.\(^11\) The details of its three-step synthesis will be described shortly (manuscript in preparation). In line with the high structural similarity of Nereid, TX-100 reduced, and TX-100 (see below), the viscosity and the propensity to foam are also comparable. Similar to other detergents, the analysis of Nereid and TX-100 reduced process intermediates can be conducted with HPLC coupled with evaporative light scattering detection (ELSD) or UV detection. The CMC of Nereid was determined at 20°C by force tensiometry, that is, sequential surface tension measurements of aqueous solutions with increasing Nereid concentration. While TX-100 reduced is commercially available, Nereid is available for evaluation under a material transfer agreement through the authors.

### 2.2 Process intermediates

Fraction II, that is, the process intermediate for immunoglobulin production at the stage prior to the virus inactivation/reduction steps, served as a model matrix for plasma-derived products. The starting material had a pH of 5.2 and was filtered through a 0.2 μm filter. The absorbance was adjusted to 28.9 AU\(_{280-320}\)/cm with 30 mM NaCl.
### Table 1: Chemical structure and CMC of tested detergents

| Structure         | CMC [mmol/L] |
|-------------------|--------------|
| TX-100            | 0.2-0.9\(^7,8\) |
| TX-100 reduced    | 0.25\(^9\)   |
| Brij C10          | 0.002\(^9\)  |
| Polysorbate 20    | 0.05\(^10\)  |
| Nereid            | 0.3-1.0 (Takeda data) |

Abbreviation: CMC, critical micelle concentration.

A model matrix for recombinant protein production was generated by diluting human albumin (25%, Baxter AG, Vienna, Austria) to 0.6 mg/mL using a buffer that contained 396 mM NaCl, 20 mM 2-(N-Morpholino)ethanesulfonic acid (MES) acid monohydrate, 10 mM CaCl\(_2\) dihydrate, and 0.099% (v/v) PS80. This matrix is representative for an intermediate of the production process of recombinant factor VIII (rFVIII; ADVATE; Baxter AG, Vienna, Austria). The starting material had a pH of 6.4 and was filtered through a 0.2 μm filter.

For an initial evaluation of PS20 with a model recombinant product, a process intermediate (supernatant of genetically modified Chinese hamster ovary [CHO] cells after ultrafiltration/diafiltration) derived from the production of
TABLE 2  Viruses and cell lines used for virus inactivation studies

| Virus        | Strain (source)                                         | Cell line for virus propagation (source) | Cell line for virus titration (source) |
|--------------|--------------------------------------------------------|-----------------------------------------|---------------------------------------|
| PRV          | Kaplan (Eberhard Karls University, Tübingen, Germany)  | Vero (ECACC, 84113001)                 | Vero (ECACC, 84113001)                 |
| X-MuLV       | pNFSTh-1 (ATCC VR-1447)                                 | Mus dunni (ATCC, CRL-2017)             | PG4 (ATCC, CRL-2032)                  |
| HIV          | HIV-1 IIIB (NIAID #398)                                 | H9 (ECACC, 85050301)                  | AA2 (NIAID, #135)                     |
| BVDV         | NaCl (ATCC VR-1422)                                     | MDBK (ATCC, CCL-22)                   | BT (ATCC, CRL-1390)                   |

Abbreviations: ATCC, American Type Culture Collection; BVDV, bovine viral diarrhea; ECACC, European Collection of Authenticated Cell Cultures; HIV, human immunodeficiency virus; MDBK, Madin Darby bovine kidney; NIAID, National Institute of Allergy and Infectious Diseases; PRV, pseudorabies virus; X-MuLV, xenotropic murine leukemia virus.

recombinant ADAMTS13 (TAK755) was obtained from the Baxter manufacturing unit. The starting material had a pH of 7.7 and was filtered through a 0.2 μm filter. The protein concentration was 0.7-0.8 mg/mL.

2.3  Virus propagation and titration

Information about all viruses, as well as cell lines, used for virus production and titration is summarized in Table 2. The panel of selected viruses reflects requirements as defined in the relevant guidelines:12,13 (a) pseudorabies virus (PRV) was included as a model for the family of *Herpesviridae*, (b) bovine viral diarrhea virus (BVDV) served as model for hepatitis C virus (HCV), which had been transmitted via plasma-derived medicines before the introduction of S/D treatment, (c) similarly, human immunodeficiency virus (HIV) was chosen due to the documented HIV contamination of coagulation factor concentrates, and (d) xenotropic murine leukemia virus (X-MuLV) served as a model for retrovirus-like particles, which are of concern due to the frequent use of rodent cell lines for recombinant protein production. Virus stocks were produced from infected susceptible cell lines essentially as described previously.14,15 For titration of virus infectivity, median tissue culture infectious dose (TCID_{50}) assays were employed using 8-fold replicates of serial half-log sample dilutions of virus-containing samples that were incubated with the respective indicator cell lines. After incubation, cytopathic effects were evaluated by microscopic visual inspection. TCID_{50} titers were calculated according to the Poisson distribution and expressed as log_{10} [TCID_{50}/mL]. Virus reduction factors (RFs) were calculated in accordance to the EU Committee for Proprietary Medicinal Products guidance.16

2.4  Virus inactivation by three-component solvent-detergent treatment

For experiments with plasma-derived products, approximately 30 mL of filtered process material was used. Throughout the entire experimental runs, the process material was kept at 17°C ± 1°C and continuously mixed by a magnetic stirrer. Spiking with the respective virus stock solution was performed at a ratio of 1:31 (v/v). Two samples—spike control (SC) and hold control (HC)—were drawn; SC was titrated immediately, whereas HC was incubated in the same cooling unit as the S/D-treated process material and titrated at the end of the respective run. As for manufacturing, the final target concentrations of PS80, TNBP, and TX-100 are 0.3%, 0.3%, and 1% (w/w), respectively. Taking the ratio of S/D components into account, different S/D mixes for virus inactivation studies were prepared by combining PS80 and TNBP with either TX-100, TX-100 reduced, Brij C10, PS20, or Nereid. For each experimental run, the weight of the spiked process material was determined to calculate the amount of S/D mix required to reach a final concentration of 5% (TX-100, TX-100 reduced, Brij C10, and Nereid) or 10% (PS20) of the concentration as specified for manufacturing. These considerably reduced final concentrations enable the demonstration of virus inactivation kinetics, a regulatory requirement,16 as opposed to virtually immediate virus inactivation at manufacturing concentrations. The detergent mix was added with a Hamilton syringe, and the actual amount of detergent added was determined by back-weighing the syringe. Samples for TCID_{50} virus titration were drawn at 1 to 2 minutes, 10 ± 1 minutes, 30 ± 1 minutes, and 59 ± 1 minutes after detergent addition.
The design of experiments for recombinant products was identical as described above, except that a temperature of 1°C ± 1°C was employed for TX-100, TX-100 reduced, Brij C10, and Nereid. In addition, PS20 was investigated at a temperature of 12°C ± 1°C and the second titration sample was drawn 5 minutes (instead of 10 minutes) after detergent addition.

For all used process materials, the cytotoxicity of the respective S/D mix, as well as any possible matrix effects on cell lines used for virus detection, was tested and taken into consideration for the calculation of RFs.

### 2.5 Virus inactivation by single-detergent treatment

For the plasma-derivative model matrix, the experimental design was identical to the experiments performed with three-component S/D mixes (see above), except that each detergent was used at a concentration corresponding to 10% of the TX-100 concentration used for manufacturing. Similarly, the design of experiments for recombinant products was identical to the corresponding three-component S/D treatments.

### 3 RESULTS

#### 3.1 Assembly of a panel of potential TX-100 surrogates

The basic concept to replace TX-100 with only minimal changes in biopharmaceutical manufacturing processes suggested looking for compounds with high structural and/or physicochemical similarity to the originally used TX-100. Consequently, candidate molecules should be nonionic, PEG-based, and have a CMC that is comparable to TX-100. Potential surrogates must, however, lack the phenyl ether moiety of TX-100 to avoid degradation products that can act as estrogen mimetic. These criteria were met by only a limited number of commercially available detergents, of which TX-100 reduced, Brij C10, as well as PS20 were chosen for further investigation (Table 1). Apart from these preexisting compounds, we also employed rational design to synthesize several novel candidate detergents. One of these was termed Nereid (4-(1,1,3,3-tetramethylbutyl)benzyl-polyethylene glycol), where, in a rather pragmatic approach, the phenyl ether moiety of TX-100 was resolved by the insertion of an extra methylene group between the aromatic ring and the oxygen atom (Table 1). Nereid is obtained in a simple three-step organic synthesis (manuscript in preparation). Altogether, Nereid completed a panel of four detergents that were evaluated for their virus-inactivating properties in a head-to-head comparative study with TX-100.

#### 3.2 Virus inactivation by three-component SD treatment

In a first set of experiments, each candidate detergent was combined with TNBP and PS80. Incubation with only 5% of the respective three-component S/D combinations at 17°C in the plasma-derivative model matrix resulted in virtually immediate and complete inactivation of PRV for the TX-100-, TX-100-reduced-, Brij C10-, and Nereid-based processes (Figure 1). In sharp contrast, virus inactivation by the PS20-based combination (even at 10% of the manufacturing specification) remained incomplete, with only 1.1 log_{10} inactivation achieved after 1 hour of incubation (Figure S1 in Supporting Information).

In the next series of experiments, we incubated X-MuLV in the recombinant protein model matrix using 10% of the respective three-component S/D combinations at reduced temperatures. Complete virus inactivation was neither observed for the detergent mix containing TX-100 nor for any other detergent mix. In accordance with the results from the plasma-derived model matrix, only minor (1.1 log_{10}) inactivation was achieved for the PS20-based process after 1 hour of incubation (Figure S1). Further investigations of PS20 were thus discontinued. Virus inactivation was also limited for TX-100 reduced (Figure 1). Interestingly, the virus RFs achieved at the end of the incubation period were still substantial for the TX-100- and the Nereid-based processes (3.2 log_{10} and 3.6 log_{10}, respectively), with slower inactivation kinetics also for Brij C10 (2.4 log_{10}). Our experiments also revealed some disadvantages of Brij C10. The waxy structure at ambient temperature, as well as the turbid nature of aqueous stock solutions, renders this compound incompatible with large-scale manufacturing. Thus, Brij C10 was not subjected to further investigations.
**FIGURE 1**  Virus inactivation of distinct three-component solvent-detergent mixes. TX-100 or potential surrogate candidates (TX-100 reduced, Brij C10, and Nereid) were combined with PS80 and TNBP to evaluate viral clearance over a time of 60 minutes. Left panels: results for PRV inactivation in the plasma-derivative model matrix under warm (17°C ± 1°C) conditions using solvent-detergent mixes at a concentration of 5% relative to manufacturing, that is, final concentrations of PS80/TNBP: 0.015%; final concentrations of other compounds: 0.05%. Right panels: results for X-MuLV inactivation in the recombinant model matrix under cold (1°C ± 1°C) conditions using solvent-detergent mixes at a concentration of 10% relative to manufacturing, that is, final concentrations of PS80/TNBP: 0.03%; final concentrations of other compounds: 0.1%. Virus inactivation performance is depicted as reduction factors (RFs), that is, the log10-transformed ratio of (a) viral load before addition of detergent mix and (b) viral load at either 1, 10, 30, or 60 minutes after addition of detergents. Open circles denote samples for which complete virus inactivation was observed, and hence, the assay detection limit was approached. Each data point is the mean of two samples that were drawn from separate experimental runs. Error bars denote standard deviation (SD; only shown if larger than the height of circles).

### 3.3 Virus inactivation by single-detergent treatment

S/D combinations based on TX-100 reduced and Nereid showed a performance largely equivalent to the original TX-100-based process, that is, substantial (recombinant/cold) or complete (plasma/warm) virus inactivation with rapid kinetics, even at concentrations dramatically lower than specified for the respective plasma or recombinant protein manufacturing processes. Consequently, we investigated whether the use of the single detergents might, in fact, be sufficient to achieve effective virus inactivation.
As shown in Figure 2, incubation with only 10% of the single detergent as would have been used for the plasma derivative model process at room temperature still resulted in virtually instantaneous and complete inactivation of HIV and BVDV, with very similar kinetics for all three detergents. Incubation with 10% of the single detergents in the cold process still resulted in approximately 2 log<sub>10</sub> X-MuLV inactivation for the TX-100- and Nereid-based processes, whereas inactivation with TX-100 reduced was insignificant. Similarly, approximately 3 log<sub>10</sub> of PRV inactivation was observed for the TX-100- and Nereid-based processes, with a lower inactivation of approximately 2 log<sub>10</sub> PRV by TX-100 reduced.

4 | DISCUSSION

The listing of TX-100 and related molecules on the REACH Annex XIV in 2017<sup>5</sup> resulted in the need for alternatives to this class of detergents in various industries. The continued use of TX-100 for established production processes beyond the “sunset date” (January 2021) will be possible to some extent (ie, by application for an authorization for continued use of TX-100 for a limited time), yet ultimately also these processes will become subject to change. In the pharmaceutical field, any significant variation of an approved manufacturing route is associated with considerable effort and costs,
increasing exponentially with the need to conduct preclinical or even clinical studies (even though the active pharmaceutical ingredient [API] itself remains unchanged). However, such animal or even human exposure can be prevented if a TX-100 surrogate represents all structural and/or physicochemical features of TX-100 as closely as possible, while being readily biodegradable, or being degraded to metabolites that would not act as estrogen-receptor agonist. Such a molecule may allow the corresponding regulatory submission to be limited to the Chemicals, Manufacturing, and Controls Section only.

Several of the commercially available detergents that have been suggested as TX-100 replacement products exhibit considerable structural differences. For instance, lauryldimethylamine N-oxide (LDAO) is a zwitterionic detergent, whereas Ecosurf EH-9 and Tergitol TNM-100X only contain relatively short branched alkyl chains as a lipophilic part of the detergent. Also, when replacing TX-100 in the classical three-component S/D mix, the latter two detergents failed to show effective virus inactivation in biotechnological process material at lower temperature (unpublished data).

PS20 is a nonionic, PEG-based detergent and was considered a promising candidate due to its low CMC (4-fold lower than the CMC of TX-100). However, PS20 poorly inactivated relevant model viruses under the conditions tested (Figure S1). TX-100 reduced has the exact same structure as TX-100 apart from having a fully hydrogenated six-membered carbon ring. The compound showed excellent virus inactivation when S/D treatment was performed at the standard temperature ($17^\circC \pm 1^\circC$) in the plasma matrix but failed to effectively inactivate X-MuLV completely in the recombinant matrix at cold temperature. Brij C10, an n-hexadecyl polyethoxylated compound, also achieved adequate virus inactivation under standard temperature conditions, but could not achieve complete virus inactivation at reduced temperatures. The results of this first screening pointed out that both TX-100 reduced and Brij C10 had acceptable virus inactivation properties in standard temperature processes but showed only moderate inactivation when tested under cold conditions.

However, Brij C10 is solid at room temperature. Solubilization requires high (i.e., $\geq 1:5$) dilution, and the resulting stock solutions exhibit pronounced turbidity. Furthermore, even diluted stock solutions tended to become solid after prolonged incubation at room temperature. Overall, these properties preclude the utilization of this detergent in routine industry operations.

The limited number of commercially available detergents that met the predefined structural requirements resulted in a rather small pool of candidates that could be screened in this study. Consequently, the plan arose to develop a tailor-made detergent that would entirely fulfill the design elements for a perfect TX-100-replacement as defined previously, without being harmful to the environment. A synthetic detergent library was established in-house, with the intention of deleting the phenol functionality of TX-100 while retaining a similar core structure. With the ultimate aim to evaluate which structural elements of TX-100 are crucial for its activity, we followed a basic structure-activity investigation that identified the detergent Nereid as an ideal candidate (manuscript in preparation). Nereid is a moon of planet Neptune, as is Triton that was discovered just over a century earlier. Furthermore, in the Greek mythology, the mother of the sea god Triton belonged to the clade of the Nereids, which were friendly sea nymphs representing the life on land and in the oceans. Therefore, the proposed name reflects well the bivalent nature of surfactants. As part of a three-component S/D mixture, the performance of Nereid was fully equivalent to TX-100, TX-100 reduced, and Brij C10 in the plasma-derivative model matrix. Notably, the virus-inactivating properties of Nereid were also similar to TX-100 in the recombinant process material under cold conditions, whereas the two other surrogate candidates were less effective. Interestingly, the inactivation of PRV was slightly more efficient for Nereid than for TX-100, whereas the opposite was observed for inactivation of X-MuLV. It is tempting to speculate that these minor variations ($<1 \log_{10}$) could be founded in minor differences in charge distribution (electron density of the aromatic ring is slightly higher for TX-100) and/or molecular geometry, probably leading to gradually distinct micellar sizes. Differences in the composition of lipid envelopes/surface proteins or virion size (PRV $> X$-MuLV) could also be contributing factors, but additional studies are needed to make final conclusions.

Over the past few decades, a plethora of biotechnological production processes has substantiated the effectiveness of the TX-100/TNBP/PS80 combination for inactivating lipid-enveloped viruses. However, to the best of our knowledge, scientific evidence explaining why exactly these three compounds need to be combined for optimum antiviral performance has never been presented. We therefore decided to investigate the virus-inactivating properties of TX-100 if used as a single detergent and to compare its performance to the most promising surrogate candidates TX-100 reduced and Nereid. Strikingly, all three compounds resulted in complete inactivation of HIV and BVDV in the plasma-derivative model matrix, with virtually indistinguishable kinetics of virus inactivation. In contrast, TX-100 reduced exhibited more modest
(PRV) or no (X-MuLV) inactivation in the recombinant model matrix under cold process conditions. The effectiveness of TX-100 and Nereid was, however, still evident even at these “worst case” conditions.

As for ecological concerns, mammalian toxicological studies and environmental fate and effect studies have been initiated to fully evaluate the safety of TX-100 reduced and Nereid. At present, a preliminary evaluation has been performed to assess persistence, bioaccumulation, and toxicity (PBT), as well as carcinogenicity, mutagenicity, and reproductive (CMR) toxicity based on predictive toxicology. These in silico analyses included both expert rule-based (Derek Nexus v6.0.0) and statistical-based (Sarah Nexus v3.0.0, OECD QSAR Toolbox v4.2) approaches that are accepted by regulatory agencies for chemical registration. Neither TX-100 reduced nor Nereid are predicted to be carcinogenic, mutagenic, or reprotoxic. Predictions also suggested that due to the lack of biodegradability, these two compounds may persist in the environment, but they were not classified as bioaccumulative or toxic. With regard to possible estrogen-mimetic activities, it should be noted that both TX-100 reduced and Nereid lack a phenyl ether moiety. The phenol group (which results from biodegradation) has been identified as being important for correct positioning of the respective small molecule within the ligand-binding domain of the estrogen receptor, and only correct positioning subsequently triggers the receptor to elicit downstream gene regulatory events. Thus, adverse endocrine effects that have been described for xenoestrogens like TX-100 degradation products (which harbor a phenol moiety) are neither expected to occur with TX-100 reduced or Nereid.

Even though the structures of Nereid and TX-100 reduced are highly similar to TX-100, possible detrimental effects on the API, as well as the detergent removal capacity, have to be assessed. In a preliminary small-scale study with rFVIII process intermediate, rFVIII concentration and rFVIII specific activity were within specifications for runs that employed the Nereid/PS80/TNBP or the TX-100-reduced/PS80/TNBP treatment and also very similar to the control run (TX-100/PS80/TNBP treatment). Residual Nereid and TX-100-reduced levels after the subsequent chromatographic purification were also in the same range as the TX-100 levels quantified for the control run. For immunoglobulin production, the possible novel S/D treatments (Nereid/PS80/TNBP or TX-100-reduced/PS80/TNBP) were compared to the current S/D treatment (TX-100/PS80/TNBP) in pilot scale studies. Analyses of the final container material—for example, for levels of neutralizing antibodies against measles virus, poliomyelitis virus, and diphtheria toxin—revealed highly similar results. Testing for Nereid and TX-100 reduced in the final container revealed a concentration below the lower limit of quantification, which again corresponds to results obtained with TX-100. In summary, it can be anticipated that the process compatibilities of Nereid, TX-100 reduced, and TX-100 are comparable.

Overall, two conclusions can be derived from the present study, which will serve as the basis for future investigations. First, Nereid and TX-100 reduced were identified as promising candidates for the substitution of the endocrine disruptor TX-100 in S/D treatment steps of biopharmaceutical processes. Second, the two other compounds of the classical S/D mix—TNBP and PS80—may be less critical than previously assumed, and thus, the future-preferred treatment could be addition of just a single virus-inactivating detergent, for example, Nereid. Such a change would result in improved effectiveness of the biopharmaceutical manufacturing process flow and its analytical monitoring, would simplify detergent removal by the downstream process, and would be viewed favorably from a health and safety perspective as TNBP is a CMR substance. Results from follow-up studies with a broader range of viruses as well as process intermediates are awaited to lend further support to this proposal.

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CONFLICT OF INTEREST
Johanna Kindermann, Michael Karbiener, and Thomas R. Kreil are employees of Baxter AG, Vienna, Austria, a member of the Takeda group of companies. Jean-Baptiste Farcet is an employee of Baxalta Innovations GmbH, Vienna, Austria, a member of the Takeda group of companies. Jean-Baptiste Farcet, Michael Karbiener, and Thomas R. Kreil are Takeda stock owners. Jean-Baptiste Farcet, Johanna Kindermann, and Thomas R. Kreil are inventors of a submitted patent application related to Brij C10, Triton X-100 reduced, and Nereid.
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SUPPORTING INFORMATION
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