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TOXICITIES OF DETERGENTS USED IN CLEANING CHEMICHALS AND HYGIENE PRODUCTS IN A TEST BATTERY OF *EX VIVO* AND *IN VITRO* ASSAYS

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ABSTRACT

Five synthetic surfactants used as detergents in cleaning chemicals and hygiene products were selected to test their In vivo and Ex vivo toxicity. The toxicity of one anionic detergent (SDS), one cationic detergent (DDDAC) and three non-ionic detergents (TWEEN 80, triton X-100, genapol X-080) was tested with boar spermatozoa (4 Ex Vivo assays) and PK-15 porcine kidney cells (4 In Vivo assays). The reference toxin used was triclosan (biocide). The results revealed a characteristic toxicity profile for each detergent. The detergents toxicity from most to least toxic was as follow: DDDAC > genapol X-080 > triclosan > triton x-100 > SDS > TWEEN 80. The results also showed that mitochondrial toxicity -measured as acceleration of glucose consumption- was detected for genapol X-080, DDAC and triclosan but not for TWEEN 80.

INTRODUCTION

The indoor air quality is interrelated with people's health /1/. Surfactants and wetting agents included in cleaning chemicals lower the surface tension of water, increasing the ability of the cleaning solution to maintain contact with solid surfaces /2/. Concern around indoor quality linking to microbial contamination, has resulted in extensive use of cleaning and antimicrobial chemicals, creating a prevalent source of indoor pollution /3,4/.

The different surfactants (non-ionic, anionic, and cationic) differ in toxicity against mammalian cells. Cationic surfactants were the only group, so far, in which sublethal mitochondrial toxicity was detected at concentrations below lethal toxicity i.e. cell lysis /5/.

Surfactants may exhibit respiratory toxicity, be harmful to mammalian cells and may have a selective impact on the indoor microbiome /6,7/. Different surfactants may differ in biological activities and these are not well known. The aim of this study was to create a versatile test battery revealing the toxicological profile, biological target and relative toxicity of common surfactants. This information would be useful for risk assessment concerning surfactants in cleaning chemicals.

MATERIALS AND METHODS

Detergents and chemicals

The detergents and chemicals used in this study are listed in Table 1.

Table 1. The list of detergents and chemicals used in this study.

Detergents	Chemical name	Type	CAS number
DDDAC*	Didecyldimethylammonium chloride	cationic	7173-51-5
Genapol X-080†	Polyethyleneglycol monalkyl ether	nonionic	9043-30-5
Triton X-100†	Polyethyleneglycol-p-is-octylphenyl ether	nonionic	9002-93-1
TWEEN 80†	Polyethylene glycol sorbitan mono oleate	nonionic	900565-6
SDS†	Sodium dodecyl sulfate	anionic	151-21-3
Reference Toxins	Activity		
Triclosan †	Mitochondrial toxin, protonophore ‡	biocide	3380-34-5
Alamethicin† (from *Trichoderma arundinaceum*)	Cation channel forming peptaibol §	mycotoxin	27061-78-5

*Merck Darmstadt, 64293, Germany. † Sigma-Aldrich, Missouri, USA. ‡ Ajao et al. /8/ § Bencsik et al. /9/

Toxicity assays

Stock solutions of 10 $mg.mL^{-1}$ were prepared, in water for the genapol X-080, triton X-100, TWEEN 80 and SDS, in methanol for DDDAC, and in ethanol for triclosan.

Ex vivo bioassays were performed using boar spermatozoa. The two criteria evaluated were spermatozoa motility inhibition (BSMI assays, sublehtal toxicity) and plasma membrane integrity (SMID assays, lethal toxicity). Both resting and motile boar spermatozoa were used in the assays, indicated as a subscripted R or M capital letter, respectively. The change of motility in the BSMI assays were determined objectively using a computed MATLAB algorithm, and subjectively, from the exact same recorded microscopic video frame. The extensive protocols are described in Castagnoli et al. /10/. SD between triplicate measurements were 13-15%. In addition, the motility inhibition provoked by DDDAC was confirmed by analysis with computer assisted semen analyser (CASA) using an Ongo Portable Semen Analyzer, (Microfluidlabs, Budapest, Hungary).

The i*n vitro* toxicity assays were performed using porcine kidney epithelial cell line (PK-15). Four criteria were evaluated, the inhibition of cell proliferation assay (ICP) mitochondrial toxicity (MITOX) evaluated as an acceleration of the glucose consumption, and cells death evaluated as inhibition of glucose consumption and inhibition or resazurin reduction /10,11/. Difference between triplicate measurements were within one of the two-fold dilution steps.

Triclosan (= mitochondrio-toxic biocide) and alamethicin (membrane channel forming mycotoxin) were used a reference toxins.

RESULTS

The toxicity of one anionic detergent (SDS), one cationic detergent (DDDAC) and three non-ionic detergents (TWEEN 80, triton X-100, genapol X-080),was investigated. Results of the *ex vivo* assays and *in vitro* assays are shown in Table 2 and Table 3, respectively.

In exposed spermatozoa the test battery measured motility inhibition (sublethal toxicity) and depletion of plasma membrane integrity (lethal toxicity) induced in resting ($BSMI_R$ and $SMIDA_R$) and motile boar sperm cells ($BSMI_M$ and $SMIDA_M$), respectively. In exposed somatic cells, PK-15, the test battery measured inhibition of cell proliferation, i.e. cytostatic toxicity, (ICP) as inhibition of resazurin reduction to the fluorescent metabolite resorufin., confirmed with microscopic inspection of cell growth. Mitochondrial toxicity (MITOX) was recorded as acceleration of glucose consumption. Cell death was measured as inhibition of glucose consumption and inhibition of reduction of resatsurin. The mitochondrio-toxic biocide triclosan and the lethal, membrane channel forming mycotoxin, alamethicin, were used as reference toxins. Results of the *ex vivo* assays and *in vitro* assays are shown in Table 2 and Table 3, respectively.

The *ex vivo* assays were faster and more sensitive (by a factor close to 10) compared to the *in vitro* assays with somatic cells. The resting sperm cells exposed at 24 °C and 24 h were more sensitive in the $BSMI_R$ assay (sublethal toxicity) than in $SMID_R$ assay (lethal toxicity). However, the toxicity endpoints obtained with the motile spermatozoa assasy were much similar (in $BSMI_M$ and $SMID_M$). Surprisingly, alamethicin, exhibited a smaller EC_{50} value after 2 hours of exposure in the $SMID_M$ assay (lethal toxicity) than after 20 minutes in the $BSMI_M$ assay (sublethal toxicity).This indicates that prolonged exposure in motile condition increased the lethal effects of this toxin (Table 2).

The most sensitive of the *in vitro* assays was the one measuring sublethal mitochondrial toxicity in cells in monolayer, the MITOX assay (Table 3), followed by the inhibition of proliferation of cells (ICP) assay. The least sensitive *in vitro* assays were the ones measuring cell death of resting cells in monolayer.

Table 2. Toxicity endpoints as µg (dry wt) ml^{-1} in ex vivo assays with resting and motile boar sperm. The solvents, methanol, ethanol and water were used as negative controls.

	EC_{50} Toxicity endpoints expressed as µg (dry wt) mL^{-1}			
Detergents	$BSMI_R$ (24h, 24°C)	$SMID_R$ (24h, 24°C)	$BSMI_M$ (20 min, 37°C)	$SMID_M$ (2h, 37°C)
TWEEN 80	10	50	10	20
Genapol X-080	3	10 (5)*	5	5
Triton X-100	1	10	1	
SDS	10	10	10	5
DDDAC	1 (0.8) †	25	0.5 (1-0.2)*	5
Reference toxins				
Triclosan	2	10	1	2
Alamethicin	0.2	0.2	5	1

*Sperm motility was analysed in parallel with by a MATLAB algorithm and by a subjective evaluation of the same microscopic video frames. *The endpoint (in brachets) obatined by subjective microscopic evaluation of motility inhibition. †endpoint (in brachets) obtained by a On go Portable Semen Analyzer.*

Table 3. Results of the In vitro bioassays performed with porcine kidney epithelial cell line PK-15.

	EC_{50} Toxicity endpoints expressed as µg (dry wt) mL^{-1}			
	Resting cells in monolayer (24-48h)			Proliferating cells in suspension (48 h)
Detergents	Cell death*	MITOX† Mitochondrial toxicity	Cell death‡	ICP‡
TWEEN 80	>100	none	100	>500
Genapol-x-080	100	5	100	30
Triton X-100				30
SDS				120
DDAC	20	1	20	0.5
Reference toxin				
Triclosan	50	10	20	8

**Inhibition of glucose consumption. † Acceleration of glucose consumption. ‡ Inhibition of resazurin reduction. The solvents used, methanol, ethanol and water were used as negative controls.*

For genapol X-080 and DDDAC,but not for Tween 80, specific sublethal mitochondrial toxicity was indicated by the small EC50 value recorded for accelerated glucose consumtion, compared with the EC50 for lethal toxicity (cell death). For genapol X-80 and DDDAC, but not for triclosan, the endpoints for mitochondrial toxicity (Table 3) were close to those measured as inhibited sperm motility (Table 2). DDDAC, but not genapol X-080 inhibited cell proliferation in very small concentration indicating an additional toxic effect of DDDAC independent of mitochondrial and plasma membrane damage.

Table 4 . Toxicological profile in 7 assays of the 5 detergents and triclosan. Toxicity is ranked from most to least sensitive assays (or 1 to 5). Number 1 indicates the most sensitive assay with the lowest EC_{50}, value, number 5 indicates the least sensitive assay, the test with the highest EC_{50} value.

	DDDAC	Genapol	Triton	SDS	Tween	Triclosan	Exposure Time	°C
$BSMI_M$	1	1	1	2	1	1	20`	37
$BSMI_R$	2	1	1	2	1	2	24 h	24
$SMIDA_M$	3	2	ND	1	2	3	2 h	37
$SMIDA_R$	4	2	3	2	3	4	24 h	24
ICP	1	3	4	3	5	3	48 h	37
CELL DEATH	5	5	ND	ND	5	5	24 h	37
MITOX	2	2	ND	ND	5	3	24 h	37
Mean of EC_{50} µg (dry wt) ml^{-1} in four tests ($BSMI_M$, $BSMI_R$, $SMIDA_R$, ICP)								
	8	10	15	36	145	12		

Table 4 summarizes the results presented in Table 2 and 3 and reveal the characteristic toxicity profile of each detergent. The profiles reflect the sensitivity of the test cells, and

the effects of exposure conditions (e.g. time and temperature) on the obtained toxicity values.

DISCUSSION

This study provides new information on the toxicity and biological targets of five commonly used surfactants and their toxicity compared to the reference toxin triclosan. The detergents toxicity from most to least toxic is: DDDAC > genapol X-080 > triclosan > triton x-100 > SDS > TWEEN 80. This information would be useful for the risk assessment of the surfactants present in cleaning chemicals and hygiene products.

The concentrations of surfactants are higher in indoor air than outdoors /8/, surfactant activity is thus an important indicator of indoor air pollution. Surfactants, with the exception of the mitochondriotoxic cationic surfactant DDDAC, have been reported to cause lethal toxicity i.e. cell lysis in vitro or hemolysis by destroying the plasma membrane integrity /5,12/. Here, this study describes more sensitive methods possibly applicable for detecting the presence of surfactants in indoor air and dust.

Sublethal toxicities – as inhibition of boar sperm and/or mitochondrial toxicity) – were detected at concentrations below cell lysis, for genapol X-080, DDDAC, triton X-100, the reference toxin triclosan, but not for SDS and the lethal toxin alamethicin. This may indicate that genapol X-080 and triton X-100 exhibit unknown sublethal toxic properties as mitochondrial toxicity. However, these results are very preliminary and needs to be confirmed by additional measurements.

Agents decreasing surface tension of water (man-made or microbially produced) very likely have an impact on water availability on indoor surfaces and mobility of substances transported by water vapour. Indoor fungi produce surfactants in extracellular vesicles, possibly acting as germinants /13/. Surfactants, man-made or microbially produced, are biologically active substances with great impact on the indoor ecosystem /10/. A method measuring surfactants in indoor air and dust by combining tensiometric measurements the toxicological profile in a battery of bioassays and identification of the bioactive substance by chemical analysis would distinguish man-made air pollutants from microbially produced substances. This information would be important for renovation work.

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