

Reprotoxic chemicals in food contact articles: The case of surfynol.

Cristina Nerín
I3A, University of Zaragoza (Spain)
cnerin@unizar.es





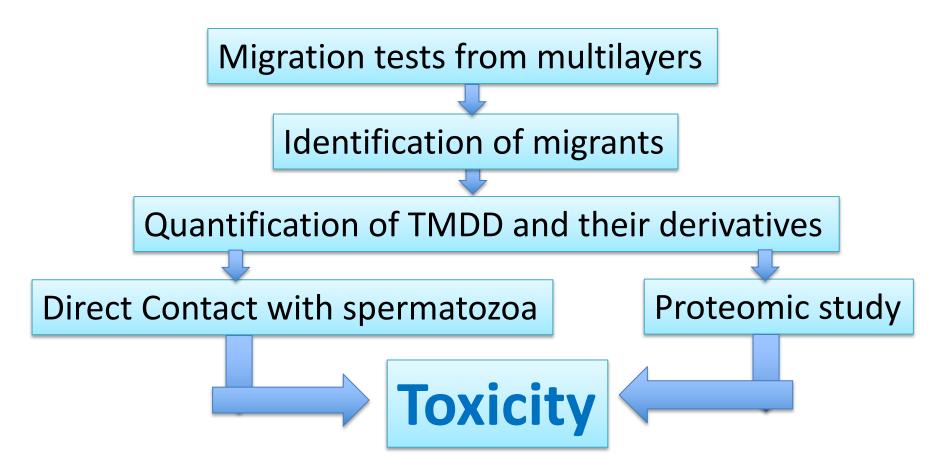
The origin

- Multilayers are very common in food packaging
- Adhesives are used to build the laminates (multilayers)
- Most of adhesive formula need surfactants
- Surfactants migrate throughout the plastic and paper layers





The study

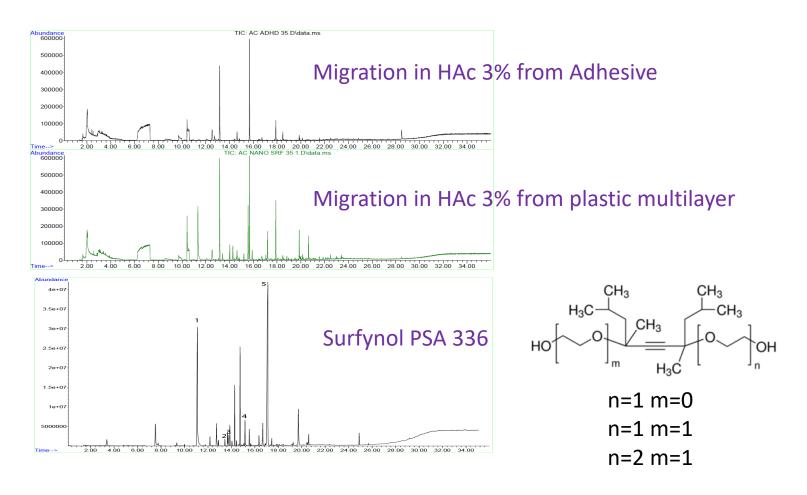


C. Nerin, et al. A common surfactant used in food packaging found to be toxic for reproduction in mammals. *Food and Chemical Toxicology Volume 113, March 2018, Pages 115-124.* https://doi.org/10.1016/j.fct.2018.01.044





Volatile migrants by HS-SPME-GC-MS



TMDD and derivatives are not degraded in acidic medium (stable for 15 days)



MIGRATION OF NON VOLATILE COMPOUNDS FROM SURFYNOL PSA 336



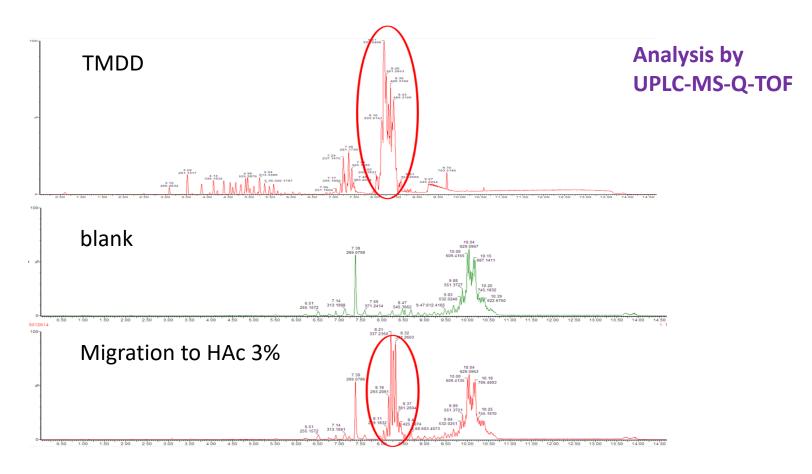


Fig. 1. UPLC-MS-Q-TOF of TMDD (A), blank (B) and acetic acid 3% (C) after exposure to plastic multilayer containing Surfynol.

Surfynol PSA 336: TMDD: 2,4,7,9-Tetramethyl-5-decyne-4,7-diol ethoxylate Class III Cramer



The migrants found

TMDD

$$\begin{array}{c|c} CH_3 & CH_3 \\ H_3C & CH_3 \\ \hline \\ HO & H_3C \\ \hline \end{array}$$

Their ethoxilated compounds

Present in **Surfynol PSA 336**



Quantitative migration

Migration values found after exposure multilayer to both acetic acid 3% and ethanol 10% in water.

Compound/migration mg/Kg	Mass Q-TOF	LOD	Ethanol 10% PE(35 μm)-adh(4 g/m²)-PET(12 μm)	Ethanol 10% PE(60 μm)-adh(4 g/m²)-PET(12 μm)	Ethanol 10% PE(90 μm)-adh(3 g/m²)-PET(12 μm)	AC 3% PE(35 μm)-adh(4 g/m²)-PET(12 μm)	AC 3% PE(60 μm)-adh(4 g/m²)-PET(12 μm)	AC 3% PE(90 μm)-adh(3 g/m²)-PET(12 μm)
1-hexanol-2-ethyl		0.001	0.005	0.003	<lod< th=""><th>0.011</th><th>0.007</th><th>0.003</th></lod<>	0.011	0.007	0.003
2,4,7,9-Tetramethyl-5-decyne-4,7-diol (sum of isomers)	249.1827	0.012	1.38	0.58	<lod< th=""><th>0.72</th><th>0.33</th><th>0.14</th></lod<>	0.72	0.33	0.14
2,4,7,9-Tetramethyl-5-decyne-4,7-diol ethoxylate n=1 m=1*(sum of isomers)	337.2353	0.012	17.07	11.08	2.90	13.40	6.42	5.67
2,4,7,9-Tetramethyl-5-decyne-4,7-diol ethoxylate n=2 m=1*(sum of isomers)	381.2614	0.012	15.89	8.81	1.68	12.70	4.77	4.40





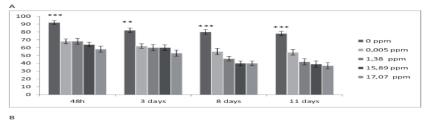
Reprotoxicity by direct contact with spermatozoa

- acrosome integrity,
- mitochondrial activity
- penetration capacity
- motility

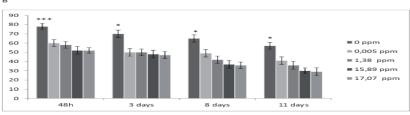




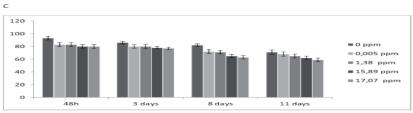
Effect of different amounts of TMDD on functionality markers of boar sperm at 48 hours, 3 days, 8 days and 11 days.



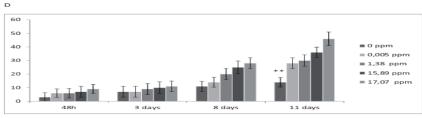




B) Progressive Motility (n = 10).



C) Viability (FITC-PNA-/IP-; n = 10).



D) Acrosome reacted (FITC-PNA+; n = 10)

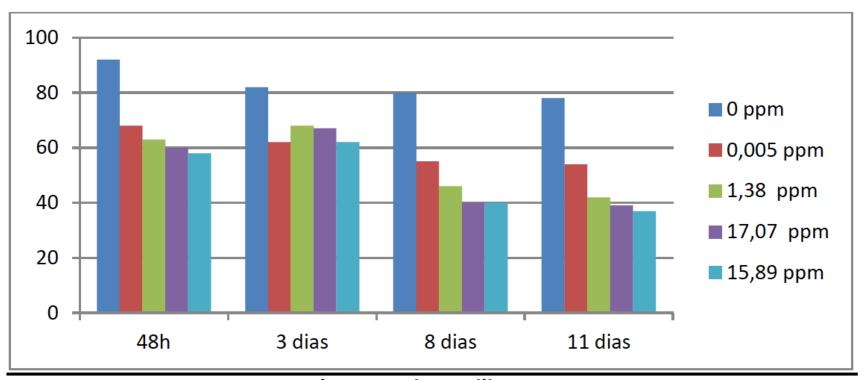
E) Mitochondrial Activity (n = 10).

Significant differences related to control: *P < 0.05, **P < 0.01, ***P < 0.001





Effect of Surfynol on reproduction



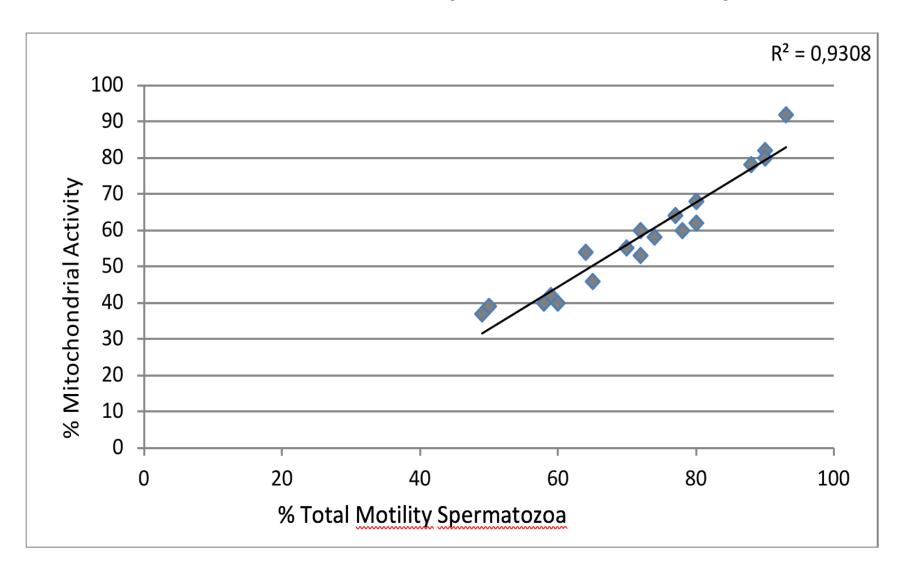
Total spermatic motility

Surfynol PSA 336 surfactant directly affects the fecundity of spermatozoa, and this effect is concentration dependent.





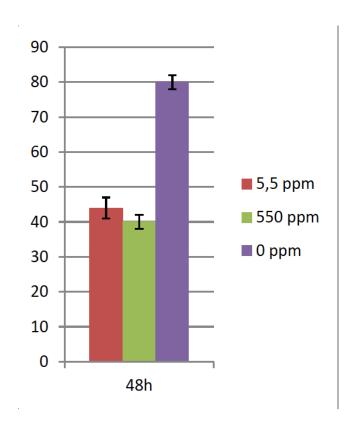
Percentage of spermatozoa with mitochondrial activity versus total motility

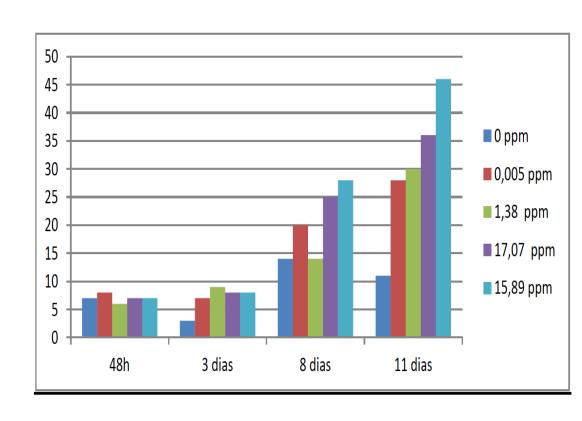






Effect of Surfynol on reproduction





Penetration test

Reacted acrosome (FITC-PNA+)%





PROTEOMIC STUDY

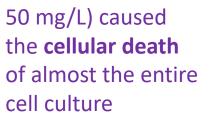
SILAC

Adaptation phase Starting culture in DMEM Media with Media with "light" AA (•) "heavy" AA (*) Begin SILAC adaptation phase Subculture cells. as needed m/zCulture desired imber of dishes, doublings Experiment phase Surfynol Control 20 mg/L 72h State A (light •) State B (heavy ★) Mix cells/lysate Optional protein or peptide fractionation analyze sample with mass spectrometry Intensity of MS signals between Intensity light and heavy peptides give relative protein abundance between cell states A and B m/z

Germinal cells of testicular embryonal carcinoma (NTERA 2 MODEL)



viability from 85% to 5% was observed with increasing concentrations of surfynol from 1mg/L to 50 mg/L respectively







Conclusions

- Reprotoxicity (male infertility) has been demonstrated by direct contact between the migrants and the spermatozoa.
- Reprotoxicity effect was confirmed by the proteomic analysis.
- Added substances (IAS, surfactant) were the cause of reprotoxicity in this case
- TMDD and their ethoxylated compounds as surfactants are quite common in food packaging materials
- Identification and quantification of migrants is extremmely important!

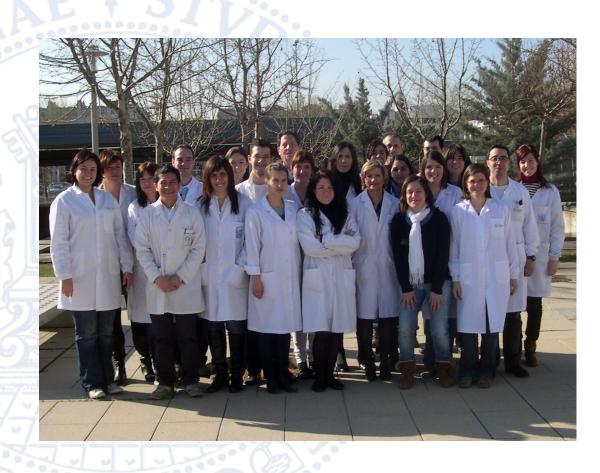




Acknowledgements

Research Projects:

- SAFEMTECH (IAPP-Marie Curie)
- SAENMA (AGL-2012, National)
- MAGAPOR
- MIGREFILMS
- MIGRESIVES (EU)
- Nafispack (EU project)
- Naturalpack (EU project)
- Nanoflexipack (National)
- FOODYPLAST (EU Project)
- R&D&i with several companies
-





Thank you very much for your attention!

cnerin@unizar.es