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A common surfactant used in food packaging found to be toxic for reproduction in mammals

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ABSTRACT

Migration from a multilayer plastic material intended for food contact showed that 2,4,7,9-tetramethyl-5-decyne-4,7-diol mixture (surfynol), used as a surfactant in the adhesive employed to build the multilayer, was transferred to water and other food simulants in contact with the plastic. When these multilayer plastics were used for containing seminal doses for artificial insemination, it was found that fertility was seriously damaged in terms of motility, acrosome integrity, mitochondrial activity and penetration capacity in the cells, thus affecting male fertility. Quantitative proteomic analysis of exposed germinal cells demonstrated the inhibition of key proteins involved in the fertilization capacity by affecting the cytoskeleton, sperm motility, the energy machinery and sperm defense mechanisms against oxidation, therefore confirming the surfactant-induced male infertility. These results open up new and interesting perspectives for the study of reprotoxicity caused by different chemicals common in our daily lives.

Significance: This paper demonstrates the toxicity for reproduction of a common surfactant used in food packaging and the scientific reasons why the sperm loses reproductive capacity in presence of this chemical. So, the surfactant affects the male fertility. The surfactant is present in many adhesives used either for building multilayer materials or to glue paper and plastic in food packaging. This is the first time that reprotoxicity is demonstrated for this compound. According to the theoretical approach Threshold of Toxicological Concern (TTC) the compound is highly toxic but experimental data did not exist so far. The study described in this paper and the results obtained open a door to further research in which male infertility caused by chemicals could be demonstrated.

1. Introduction

In 2010, a migration study of several food packaging materials reported a concentration of 621.0 μg/kg food of 2,4,7,9-tetramethyl-5-decyne-4,7-diol (TMDD) in solid simulant Tenax (Canellas et al., 2010). No experimental data about the toxicity of TMDD were available, but the theoretical prediction according to the theoretical approach of the threshold of toxicological concern (TTC) (Barlow, 2005) classified this compound as Cramer class III, the highest toxicity level, for which the value of 90 μg/kg food should not be surpassed. According to the document written by Susan Barlow (2005), TTC is a principle that refers to the establishment of a generic human exposure threshold value for chemicals below which there would be no appreciable risk to human health. The concept proposes that such a value can be identified for many chemicals, including those of unknown toxicity when considering their chemical structures, such as the presence of aromatic rings, double or triple bonds, heterocycles and heteroatoms. The use of the TTC principle would eliminate the necessity of extensive toxicity testing and safety evaluations when human intakes of a chemical are below a certain level of concern. Obviously, those compounds taken in over the proposed limits and theoretically classified as toxic should be evaluated. Toxicity prediction for humans can be also valuable to select potential damage of chemicals in other mammals. Then, the migration of chemicals from materials used for artificial insemination should be taken with care, as all chemicals transferred from the packaging will be directly introduced into the animal. Previous studies carried out *in vivo*

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with other migrants (Nerin et al., 2014) demonstrated the importance of chemicals in contact with sperm cells. Justification of those packaging materials with TMDD or its mixture called surfynol, present in the market in 2010, was based on the small amount of adhesive used to build such materials. However, the market evolved, and packaging materials currently can contain more of this surfactant, as polyurethane adhesives are being substituted for aqueous-based in many plastic applications. Thus, the whole surface of the plastic layer will be coated with adhesive to glue the additional layers in the laminated structure, and the presence and concentration of this surfactant can be very high.

Surfynol is a common surfactant used in coatings, inks and adhesives employed in many food-packaging applications. Industrial surfynol is a mixture of ethoxylated compounds, the major compounds being those with 1 and 2 ethoxy units. The surfactant is not applied on the surface in direct contact with the food but behind the plastic layer in multilayer (laminated) structures. However, migration occurs through the different layers, either paper or plastic, to both solids and liquids in contact with the packaging, as was demonstrated in several publications (Aznar et al., 2011; Canellas et al., 2015; Felix et al., 2012; Isella et al., 2013; Nerin et al., 2014; Vera et al., 2011, 2013). Many different formulas of aqueous dispersion adhesives, which are probably the new generation of environmentally friendly adhesives, use this surfactant because of its efficiency, availability and price. An in-depth study was carried out on some multilayers containing this surfactant in the adhesive used to build flexible material. It was found that seminal doses packaged in plastic bags containing this surfactant in the structure caused the inactivation and lack of fertility of spermatozoa. For this reason, migration of this surfactant to aqueous and ethanol solutions and its toxic effect on spermatozoa from mammals were investigated. The cellular model NTERA2, consisting of germinal cells of testicular embryonal carcinoma, was used for the toxicity test. In addition, to obtain a deeper insight into the biomolecular mechanisms underlying the potential toxicity of surfynol, a quantitative proteomic approach named SILAC was carried out. Stable isotopic labeling by amino acids in cell culture (SILAC) is one of the most widely used alternatives for relative protein quantitation due to its high accuracy and because it offers the possibility for the identification and quantitation of proteins within the same experiment. SILAC involves the addition of ^{12}C- and ^{13}C-labeled lysine and arginine to the growth media of separately cultured cells, giving rise to cells containing “light” or “heavy” proteins, respectively, which are further identified and quantified by mass spectrometry (Luque-Garcia et al., 2011). The reproduction capacity and integrity of spermatozoa were also evaluated by studying acrosome integrity, mitochondrial activity and penetration capacity. All experimental data confirmed the negative effect on spermatozoa of this compound. On the one hand, the present study demonstrates that direct exposure of chemicals to living cells, such as spermatozoa, is a feasible and cheap method for reprotoxicity studies concerning male fecundity. On the other hand, this research notes the risk that untested packaging materials can represent for assisted reproduction, either in animals or humans. The results obtained are shown and discussed.

2. Materials and methods

2.1. Ethical statement

No human participants were involved in the research. The use of animals is limited to collecting semen from male Spanish boars and harvesting of ovaries from pigs at the official slaughterhouse of the Council. The authors confirm that all methods were carried out in accordance with relevant guidelines and regulations. The sampling procedures complied with *Ethics* Committee for Animal Research of the University of Zaragoza.

2.2. Samples

An aqueous dispersion of adhesive containing 7% surfynol, a mixture of 2,4,7,9-tetramethyl-5-decyne-4,7-diol (TMDD) monomer and its ethoxylated polymers as a surfactant was used to glue a 35, 60 or 90 μm low-density polyethylene (LDPE) layer to a 12 μm polyethylene terephthalate (PET) layer. Surfynol was provided by Samtack (Barcelona). Different multilayers were obtained with 3 and 4 g/m^2 of grammage for adhesive in the multilayer. Ultra-high-performance liquid chromatography with quadrupole and time of flight mass spectrometry (UPLC-MS-Q-TOF) was used for analysis of surfynol, the adhesive and food simulants after the exposure to plastic multilayers for 10 days. A standard of the TMDD monomer (Sigma-Aldrich (Madrid, Spain) was used as a calibrant for quantitative purposes.

2.3. Migration tests

Bags of 10 cm × 5 cm were manufactured with the multilayer laminate described above and filled with 30 mL of ethanol 10% and acetic acid 3%. Three replicates of both simulants were prepared. These samples were kept in the oven at 60 °C for 10 days according to Directive 10/2011/EU. Finally, the extracts were analyzed by UPLC-MS-Q-TOF using the method explained above.

2.4. Analysis by UPLC-MS-Q-TOF

Chromatography separation was carried out in an AcquityTM system. The column used was an Acquity UPLC BEH C18 column of 17 μm particle size (2.1 mm × 100 mm) from Waters (Milford, MA, USA). Methanol and water were used as the mobile phase, both with 0.1% formic acid. The column flow and column temperature were 0.3 mL/min and 40 °C, respectively. The gradient was 5–95% methanol and 0.1% formic acid (0–25 min), and the volume of sample injected was 5 μL.

An API source (atmospheric pressure ionization) with an electrospray interface (ESI) coupled to a Xevo G2 mass spectrometer, which consisted of a hexapole, a quadrupole, a collision cell and a time of flight analyzer (QTOF) from Waters (Milford, MA, USA), was used. Electrospray was operated in positive (ESI+) mode with two different cone voltages, 30 and 70 V, and negative (ESI-) mode to detect as many compounds as possible. The corona voltage was 2.5 kV for (ESI+) and 0.5 kV for (ESI-). Other MS parameters were as follows: the mass range was from 10 to 1000 Da, the source temperature was 150 °C, the desolvation gas temperature was 450 °C, and the desolvation gas flow was 650 Lh-1. MSE mode was selected for the acquisition. The collision ramp energy was selected from 15 to 40 V. MassLynx v.4.1 software (Waters, Milford MA, USA) was used to analyze the samples.

To determine the migrants coming from the laminate in each simulant, a comparison between the chromatograms of the laminate and their respective blanks was carried out. The selected peaks coming only from the laminate were identified using Elemental Composition, Mass Fragment TM and MSE software as well as the chemical databases ChemSpider [www.chemspider.com] and Scifinder [scifinder.cas.org]. Using these tools, the potential candidates were selected. Confirmation was done with the pure standards analyzed under the same conditions.

2.5. Sperm collection

Semen was manually collected by the double gloved hand technique, using a gauze filter to remove the bulbourethral gland gel secretion. All ejaculates were collected in different Spanish boar studs, diluted 1:10 in commercial boar semen extender Duragen® with antibiotics and then immediately sent to Magapor SL quality control laboratories. Twenty ejaculates were collected from 10 different animals. The ejaculates were used individually. Samples were collected into a prewarmed insulating collection flask, within which was a 450 mL