UNIVERSIDAD AUTÓNOMA DE SAN LUIS POTOSÍ

FACULTAD DE CIENCIAS QUÍMICAS, INGENIERÍA,
MEDICINA Y CIENCIAS SOCIALES Y HUMANIDADES

PROGRAMA MULTIDISCIPLINARIO DE POSGRADO EN
CIENCIAS AMBIENTALES

TESIS QUE PARA OBTENER EL GRADO DE

DOCTORA EN CIENCIAS AMBIENTALES

REDUCCIÓN DE COMPUESTOS TÓXICOS PRESENTES EN DETERGENTES, UTILIZANDO NUEVAS MATERIAS PRIMAS ECOLÓGICAS.

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Enero 2021

CRÉDITOS INSTITUCIONALES

PROYECTO REALIZADO EN:

FACULTAD DE INGENIERIA
DE LA UNIVERSIDAD AUTÓNOMA DE SAN LUIS POTOSI

CON FINANCIAMIENTO DE:

PROMEP 031-2013

A TRAVÉS DEL PROYECTO DENOMINADO:

Tratamiento de aguas residuales de la industria

AGRADEZCO A CONACyT EL OTORGAMIENTO DE LA BECA-TESIS

Becario No. 618516

EL DOCTORADO EN CIENCIAS AMBIENTALES RECIBE APOYO A TRAVÉS DEL PROGRAMA NACIONAL DE POSGRADOS DE CALIDAD (PNPC)

CRÉDITOS INSTITUCIONALES

PROYECTO REALIZADO EN:

LABORATORIO DE SALUD TOTAL DEL CENTRO DE INVESTIGACIÓN APLICADA EN AMBIENTE Y SALUD (CIAAS) DE LA FACULTAD DE MEDICINA – COORDINACIÓN PARA LA INNOVACIÓN Y APLICACIÓN DE LA CIENCIA Y TECNOLOGÍA (CIACYT) DE LA UNIVERSIDAD AUTÓNOMA DE SAN LUIS POTOSÍ.

CON FINANCIAMIENTO DE:

CONACYT A1-S-28176

A TRAVÉS DEL PROYECTO DENOMINADO:

PROYECTO FOSEC SEP-INVESTIGACIÓN BÁSICA 2017-2018 CON NÚMERO A1-S-28176: SÍNTESIS Y EVALUACIÓN DE POLÍMEROS DE IMPRESIÓN MOLECULAR PARA LA DETERMINACIÓN ANALÍTICA DE CONTAMINANTES EMERGENTES EN MATRICES AMBIENTALES

EL DOCTORADO EN CIENCIAS AMBIENTALES RECIBE APOYO ATRAVÉS

DEL PROGRAMA NACIONAL DE POSGRADOS DE CALIDAD (PNPC)

DEDICACIÓN

Dedico esta tesis a mi esposo y mi hijo que son mi motor.

A mis papás que siempre me han apoyado y enseñado a ser como soy.

A mis hermanas que siempre están conmigo.

AGRADECIMIENTOS

En primer lugar, quiero agradecer a mi esposo por acompañarme y apoyarme desde siempre en cualquier proyecto que se me ocurre, te amo. A mi hijo Andrés por ser mi motor y hacerme saber que todo siempre de alguna forma va a estar bien.

Quiero agradecer a mis papás y a mis hermanas, que, aunque no estemos siempre de acuerdo, se que puedo contar con su apoyo siempre sin importar lo que sea. Los quiero mucho.

Agradezco a mi Director de Tesis, el Dr. Luis Armando Bernal Jácome, por la confianza depositada en mí y por su continua asesoría y acompañamiento. Así como a mi Comité Tutelar (Dra. Ma. Catalina Alfaro de la Torre y el Dr. Alfredo Ávila Galarza) por las continuas revisiones y aportaciones para el desarrollo de esta tesis.

Un especial agradecimiento a todos los que forman parte del Laboratorio de Salud Total del Centro de Investigación Aplicada en el Ambiente y Salud (Dr. Rogelio Flores, Maribel, Lorena, María, Toño, Lulú) que me abrieron sus puertas y fueron parte esencial para el desarrollo experimental de esta tesis y la publicación de artículos de investigación.

Al Dr. Jordi Morató de la Universidad Politécnica de Catalunya que me recibió y apoyó con el desarrollo de investigación en mi estancia de investigación, así como a los compañeros de laboratorio que colaboraron conmigo (Nury, Lorena, Angela).

¡MUCHAS GRACIAS A TODOS!

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RESUMEN

Los contaminantes emergentes (CEs) representan un significante riesgo para la salud humana, ecológica y ambiental. Aunque existe progreso en la implementación de monitoreo en matrices ambientales, efectos humanos, legislación y control, aún existen problemas asociados con el sesgo regional y los tipos de CEs comúnmente evaluados, lo que puede subestimar el riesgo para la salud. Los Alquilfenoles etoxilados (principalmente el Nonilfenol Etoxilado) son considerados como CEs ya que son disruptores endocrinos y son ampliamente utilizados en detergentes. Por lo tanto, estos son uno de los contaminantes más comunes en agua causando problemas ambientales como generación de espuma y efectos tóxicos en la biota. Esta tesis identifica las actuales deficiencias de investgiación del Nonilfenol (NF) ya que actualmente no hay regulaciones en la región de América Latina. Adicionalmente, se desarrolló un método analítico cromatográfico utilizando micro extracción en fase sólida (SPME) combinado con cromatografía de gases acoplado a masas (GC-MS) para la extracción y cuantificación del 4-NF en muestras de agua. Se realizó un monitoreo en agua recreacional, agua residual y agua potable, así como una evaluación de riesgos en humanos por la exposición de NF a través del consumo de agua. Los resultados muestran que las concentraciones de 4-NF encontradas pueden causar impactos ambientales potenciales y pueden representar un riesgo a la salud pública. Por lo tanto, la presente tesis propone y evalúa la sustitución del nonilfenol etoxilado (NFE) con un alquil poliglucósido (APG). Los resultados muestran que el APG puede ser un sustituto adecuado debido a que ofrece propiedades similares o mejores que los detergentes formulados con NFE, eliminando los riesgos de exposición asociados. La importancia de esta investigación recae en el hecho de que la Convención de Rotterdam ha catalogado al NF como un compuesto severamente restringido, por lo que es necesario establecer medidas para su restricción y cambio a tecnologías sustentables.

Palabras clave: Nonilfenol etoxilado, Nonilfenol, Alquil poliglucósido, América Latina, Micro-extracción en fase sólida, riesgos ecológicos y de salud.

ABSTRACT

Emerging pollutants (EPs) represent a significant risk to human, ecological and environmental health. Although progress has been made in establishing monitoring in environmental matrices, health effects, legislation and control, there are still problems associated with regional bias and the types of EPs commonly assessed, which may underestimate the risk to health. alquilphenol ethoxylates (mainly nonylphenol ethoxylate) are considered as EPs as they are endocrine disruptors and they are widely used in detergents. Therefore, detergents are one of the most common pollutants in water causing environmental problems like foam generation and toxic effects in biota. This thesis identifies the current research deficiencies for Nonylphenol (NP) as currently there are not any regulations in Latin American region. In addition, a chromatographic analytical method was developed using solid phase microextraction (SPME) combined with gas chromatography-mass spectrometry (GC-MS) for the extraction and quantification of 4-NP in water samples. A monitoring in recreational water, wastewaters and drinking water was performed as well as a risk assessment in humans for NP exposure through water intake. Results show that concentrations of 4-NP found could cause a potential environmental impact and may represent a public health risk. Therefore, this thesis proposes and evaluates the substitution of nonylphenol ethoxylate (NPE) with alkyl poliglucoside (APG). Results show that APG may be an adequate substitute because it offers similar and/or better properties than detergents formulated with NPE, eliminating associated exposure risks. The importance of this investigation lies in the fact that the Rotterdam Convention has catalogued NP as a severely restricted compound, so it is necessary to establish measures for its restriction and change to a sustainable technology.

Keywords: Nonylphenol ethoxylate, Nonylphenol, Alkyl Polyglucoside, Latin America, Solid-phase micro-extraction, environmental and health risks.

INTRODUCCIÓN

Las Conferencias de las Naciones Unidas sobre el Medio Ambiente y el Desarrollo (UNCED, por sus siglas en inglés) se han realizado desde 1972 para tratar temas importantes, relacionados con el cuidado del medio ambiente. Sin embargo, la conferencia celebrada en Río de Janeiro, Brasil, en 1992 fue la que marcó una pauta importante debido a lo ambicioso de los temas y las metas que se abordaron. Dicha reunión trató temas relacionados con el ambiente y el desarrollo sustentable, surgiendo nuevas políticas y principios generales de acción para gobiernos y empresas privadas. Desde Río 1992 ha tomado mayor importancia la preservación y cuidado del ambiente y se han incrementado las regulaciones gubernamentales en algunos países, con el propósito de controlar y asegurar el buen desempeño ambiental por parte de las empresas.

Actualmente, la contaminación de mantos acuáticos es un tema de relevancia creciente, siendo una de las causas principales el desecho de detergentes domésticos e industriales. Los surfactantes son componentes básicos en la formulación de detergentes, que contribuyen de manera importante a la contaminación de aguas residuales de todo tipo. Los detergentes comúnmente contienen una mezcla de alquil fenol etoxilados (AFEs) con diferente longitud de cadena e isómeros, predominando los grupos nonil y octil. El nonilfenol etoxilado (NFE) es un líquido viscoso de color amarillo que proviene del proceso de refinación del petróleo y su preparación técnica es una mezcla compleja de varios isómeros. Por ser un surfactante con un desempeño excepcional es ampliamente utilizado en la industria como materia prima para detergentes, emulsificadores, lubricantes, agentes dispersores, entre otros. Debido a su bajo precio y a sus excelentes propiedades detergentes, particularmente útiles para remover grasas y aceites, su aplicación en detergentes representa el 80% de la demanda de este surfactante y es el más empleado en la actualidad y desde hace más de 50 años.

La gran demanda industrial por surfactantes ha incrementado la presencia del NFE como contaminante de manera universal, integrándose al medio ambiente principalmente por la descarga de desechos y por las plantas tratadoras de aguas. En condiciones aerobias, el proceso de biodegradación del NFE comienza con el acortamiento de su cadena para después oxidarse, formando su metabolito nonilfenol (NF). El NF es persistente a la degradación y se ha establecido que es, aproximadamente, 10 veces más tóxico que su precursor, ya que es capaz de imitar el desempeño de importantes hormonas, resultando en la disrupción de diferentes procesos endocrinos, interfiriendo con las señales que controlan la fisiología general de todos los organismos. Como consecuencia, algunos países han regulado el uso del NFE en la industria. La Unión Europea restringe su uso con la Directiva 2003/53/EC, la cual establece que no se pueden comercializar o usar como sustancias o constituyentes de preparados en concentraciones iguales o superiores al 0.1% en masa de NF o 1% en masa de NFE, y bajo la Directiva 775/2004 (02/2076) se prohibió su uso para la elaboración de plaguicidas. El Convenio de Rotterdam, lo clasifica como químico prohibido o rigurosamente restringido. La Agencia de Protección Ambiental (EPA por sus siglas en inglés) recomienda que las concentraciones de NF en agua dulce sean menores a 6.6 µg L^{-1} y en agua salada menores a 1.7 µg L^{-1}, y el Acta Canadiense de Protección Ambiental los agrega en 1999 a su lista de sustancias tóxicas.

Debido a los importantes efectos ambientales y de salud se propone la sustitución del NFE en las formulaciones de detergentes como medida posible para minimizar los riesgos ambientales presentes actualmente. El presente trabajo tiene como objetivo determinar las propiedades fisicoquímicas de materias primas en detergentes para su evaluación general y de desempeño. Adicionalmente, se estandariza y valida una metodología para la identificación del 4-nonilfenol en diferentes fuentes de agua, para realizar un monitoreo en México, países de Europa Central y Reino Unido y así determinar las implicaciones de salud y ambientales de la exposición de dicho compuesto.

La presente tesis se presenta en capítulos formados por artículos científicos publicados y/o en revisión. En el Capítulo 1 se realiza una revisión bibliográfica partiendo de los contaminantes emergentes (CEs) y tomando como caso de estudio el NF. En el Capítulo 2 se presenta la estandarización y validación de un método analítico para la identificación y cuantificación del 4-NF y se comprueba su factibilidad realizando un monitoreo en muestras de aguas recreacionales, aguas residuales y agua potable en México. El Capítulo 3 muestra una evaluación de riesgos en humanos por la exposición de NF a través del consumo de agua. Por ultimo, el Capítulo 4 propone y evalua la sustitución del NFE en detergentes como desarrollo sustentable tecnológico.

INTRODUCTION

The United Nations Conference on Environment and Development (UNCED) has been celebrated since 1972 to discuss and address important issues related to the environment. However, the conference held in Rio de Janeiro, Brasil in 1992 set an important guideline due to the ambitious goals established. This conference discussed environmental topics and sustainable development, emerging new policies, and general principles of action for governments and private businesses. Since Rio 1992 the preservation and environmental care have taken on greater importance. Besides, new regulations have been established in several countries to control and assure environmental care and development.

Currently, water pollution is a growing concern. Detergent disposal is one of the main causes of this contamination because of the surfactants used in domestic and industrial detergents. Usually, detergents contain a mixture of alkylphenol ethoxylates (APEs) with different chain lengths and isomers, mainly nonyl and octyl groups. Nonylphenol ethoxylate (NPE) is a viscous yellow liquid that comes from the oil refining process and its technical preparation is a complex mixture of several isomers. NPE is commonly used in detergents, emulsifiers, lubricants, dispersing agents, above others, due to its exceptional performance. Its application in detergents represents 80% of its demand because of its low price and excellent detergent properties to remove greases and soil. Therefore, NPE is the most used surfactant currently and for more than 50 years.

The great industrial demand for surfactants has increased NPE presence as pollutant integrating into the environment through waste discharges and water treatment plants. In aerobic conditions, NPE biodegrades into a shorter chain to oxidize forming nonylphenol (NP). This metabolite is more persistent to degradation and is 10 times more toxic than its precursor because of its capability to mimic important hormones resulting in the disruption of different endocrine processes interfering with signals that control the physiology of multiple organisms. Thus, some countries have regulated the use of NPE in the industry. The European Union (EU) restricts its use with Directive 2003/53/EC that establishes that NPE may not be placed on the market or used as a substance or constituent of preparations in concentrations equal or higher than 0.1% by mass of NP or 1% by mass of NPE, while Directive 775/2004 (02/2076) prohibited its use in pesticides. Rotterdam Convention classifies it as a prohibited chemical. The Environmental Protection Agency recommends concentrations of NP in freshwater lower than 6.6 µg L-1 and saltwater lower than 1.7 µg L-1, and the Canadian Environmental Protection Act considers it as a toxic substance.

Due to the important health and environmental risks that NP represents this investigation proposes the substitution of NPE in detergent formulations as a possible measure to minimize adverse effects. The objective of this study is to determine the physico-chemical properties of raw materials in detergents for general and performance evaluation. Also, an analytical method is developed for the identification of 4-NP in different water samples to monitor water in Mexico, countries in Central Europe, and the United Kingdom and determine health and environmental implications from the exposure of this compound.

This thesis is presented in chapters formed by scientific papers published and/or under review. Chapter 1 presents a bibliographic review starting from emerging pollutants (EPs) and taking NP as a case study. Chapter 2 describes the standardization and validation of an analytic method for the identification and quantification of 4-NP and its feasibility is demonstrated with monitoring of water samples (recreational, wastewaters, and drinking water) in Mexico. In Chapter 3 a risk assessment is performed in humans for NP exposure through water intake. Finally, Chapter 4 proposes and evaluates NPE substitution in detergents as sustainable technological development.

JUSTIFICACIÓN

La producción global de químicos ha crecido de 1 millón de toneladas en 1930 a 400 millones de toneladas en el 2004. Como la mayoría de estos químicos comenzaron su producción antes de 1981 no se han sujetado a los requerimientos de evaluación ambiental. Por lo tanto, únicamente el 3% de los 2500 químicos de alto uso industrial son correctamente evaluados (Soares et al, 2008). Dentro de estos químicos se encuentran los surfactantes que son compuestos producidos en grandes cantidades y utilizados en el hogar y la industria en procesos de limpieza. Por lo mismo, se consideran como uno de los principales contaminantes orgánicos de origen antropogénico. Después de su uso son usualmente desechados en los drenajes para su tratamiento en plantas tratadoras de agua donde los surfactantes son removidos parcial o completamente por separación o biodegradación. Los porcentajes de remoción de surfactantes en plantas tratadoras de agua presentan un rango del 95 al 99% (Petrovic y Barceló, 2004). Aún cuando la remoción es alta, el principal problema ambiental es debido a la formación de metabolitos provenientes de los surfactantes (ej. nonilfenol). Dichos metabolitos son resistentes a la degradación y son de especial interés debido a que las concentraciones que reciben las aguas superficiales son tales que pueden causar efectos adversos en organismos acuáticos, suelos, aire e incluso en humanos. La exposición directa e indirecta en animales y humanos al NF es un problema de creciente relevancia. Hay evidencia de que este puede ser eliminado del organismo, sin embargo, la exposición continua a esta sustancia puede dificultar su eliminación de manera eficiente. Mientras que los organismos pueden evolucionar en respuesta a diferentes tipos de contaminación, los estrógenos ambientales pueden afectar a la diferenciación sexual la cual es un componente necesario para la evolución. Este hecho por sí solo hace que la contaminación por el NF sea un problema importante. Aunado a esto existen múltiples estudios que indican que el NF representa un riesgo potencial importante tanto para el medio ambiente como para la salud de los humanos (Vazquez-Duhalt et al, 2005).

Una de las principales vías de exposición es a través del consumo de alimentos y agua lo que causa bioacumulación y biomagnificación. Estudios previos en animales han mostrado efectos por exposición en concentraciones desde 1 µg L^{-1} hasta >195 µg L^{-1} (Lussier et al., 2000; Scaia et al., 2019; Tabassum et al., 2017), sin embargo, los efectos en humanos son aún debatidos y requieren de más investigación. Algunos estudios sugieren que los efectos por la exposición al NF incluyen una disminución en el conteo espermático, incremento en cáncer de próstata, mama, de ovarios y testicular, malformaciones reproductivas, deficiencia inmune, efectos neurológicos, desarrollo intelectual disminuido en niños y efectos psicológicos siendo la población mas vulnerable los fetos e infantes (Bolong et al., 2009; Tijani et al., 2016). A pesar de esto, la concentración prevista sin efecto no ha sido establecida por que no hay información toxicológica suficiente y el mecanismo específico en los organismos es todavía poco entendido (Bakke, 2003).

La sustitución del NFE en las formulaciones de detergentes es una medida posible para minimizar potenciales riesgos ambientales y de salud. Es posible considerar el alquil poliglucósido debido a que según la literatura ofrece propiedades físico-químicas y ecológicas similares o mejores que las del NFE. Algunos de los beneficios esperados en la industria y sociedad podrían ser una disminución en el consumo de surfactantes sintéticos (reflejado en beneficios ambientales como la disminución de emisiones de CO_2), menor consumo de combustibles fósiles y una menor preocupación para la disposición final de los productos.

JUSTIFICATION

Global chemical production has grown from 1 million tons in 1930 to 400 million tons in 2004. Because most of these chemicals started their production before 1981 they are not environmentally evaluated, therefore, only 3% of the 2500 industrial chemicals are well evaluated (Soares et al, 2008). Surfactants are compounds produced in large quantities used for cleaning processes at home and in industry. Thus, it is considered as one of the main organic pollutant of anthropogenic origin (Reference). Detergents after their use are commonly disposed in the drain and in water treatment plants surfactants are partially or completely removed by separation or biodegradation. The removal percentage of surfactants in water treatment plants presents a range from 95 to 99% (Petrovic and Barceló, 2004). Even when it presents a high removal rate, the main environmental problem is due to the formation of metabolites formed from surfactants (i.e. nonylphenol). These compounds are resistant to degradation and are of special interest because of the high concentrations in surface water and the adverse effects on aquatic organisms, soil, air, and humans (Reference). Direct and indirect exposure in animals and humans to NP is a problem of growing concern. There is evidence that it may be eliminated from the organism, however, continuous exposure may difficult its efficient elimination (Reference). While organisms may evolve in response to different types of pollution, environmental estrogens may be affected in the sexual differentiation. Additionally, previous studies show that NP exposure represents an important potential risk to the environment and human health (Vazquez-Duhalt et al, 2005).

The main exposure pathway for humans is through food and water intake which leads to bioaccumulation and biomagnification. Effects of NP exposure in animals have been previously reported in concentrations from 1 µg L^{-1} up to >195 µg L^{-1} (Lussier et al., 2000; Scaia et al., 2019; Tabassum et al., 2017), however, effects in humans are still debated and require more investigation. Some studies suggest that exposure effects include decreased sperm count, an increase in the prostate, mama, ovarian and testicular cancer, reproductive malformations, immune deficiency, neurological effects, poor intellect development in children, and psychological effects being the more vulnerable populations fetuses and newborns (Bolong et al., 2009; Tijani et al., 2016). Despite this, the predicted no-effect concentration (PNEC) of NP has not been established because there is not enough toxicity data and the specific mechanism in organisms is still unclear (Bakke, 2003).

NPE substitution in detergents is a possible measure to minimize potential environmental and health risks. Alkyl Polyglucosides may be considered as a substitute because, according to the literature, it presents physical and chemical properties similar or better than NPE. Some of the expected benefits in the industry and society are the decrease in synthetic surfactant consumption (reflected in environmental benefits like a decrease in CO_2 emissions), lower consumption of fossil fuels, and less concern with waste disposal.

OBJETIVOS

OBJETIVO GENERAL

Determinar la posible sustitución del Nonilfenol Etoxilado en las formulaciones de detergentes como medida posible para minimizar los riesgos ambientales y de salud presentes actualmente.

OBJETIVOS ESPECÍFICOS

1. Estandarizar y validar una metodología para la identificación y cuantificación del 4-nonilfenol (4-NF) en muestras de agua.
2. Realizar un monitoreo en muestras de agua en México, países de Europa Central y Reino Unido
3. Determinar las implicaciones de salud en humanos por la exposición del nonilfenol.
4. Determinar las propiedades fisicoquímicas de materias primas ecológicas en detergentes para su evaluación general y de desempeño para establecer la posible sustitución del NFE en detergentes.

OBJECTIVES

GENERAL OBJECTIVE

Determine the possible substitution of Nonylphenol Ethoxylate in detergents as a possible measure to minimize current environmental and health risks.

SPECIFIC OBJECTIVES

1. Standardize and validate a method for the identification and quantification of 4-nonylphenol (4-NP) in water samples.
2. Conduct monitoring in water samples from Mexico, countries in Central Europe, and the United Kingdom.
3. Determine health implications in humans for nonylphenol exposure.
4. Determine physical and chemical properties of natural surfactants in detergents for a general and performance assessment to establish the possible substitution of NPE in detergents.

1. THEORETICAL FRAMEWORK: EMERGING POLLUTANTS (EPs) IN LATIN AMERICA: A CRITICAL REVIEW OF UNDER-STUDIED EPs, CASE OF STUDY – NONYLPHENOL –

Science of the Total Environment 726 (2020) 138493

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Review

Emerging pollutants (EPs) in Latin América: A critical review of under-studied EPs, case of study -Nonylphenol-

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ABSTRACT

The growing scarcity of water resources is one of the most critical environmental problems faced in several regions in the world. A new type of pollutants defined as “emerging pollutants” significantly affects water quality and causes potential problems in public health and environmental security. Due to their recent detection and low concentrations found there is a gap in the knowledge about their occurrence, fate, behavior, risk assessment, and ecological and human effects mainly in developing countries like those in Latin America. This review gives a comparison on emerging pollutant occurrence as well as current regulations in this regard, in addition, nonylphenol is taken as a case study. Nonylphenol is a degradation product of nonylphenol ethoxylate, which is a surfactant widely used in the manufacture of detergents in Latin America; due to its lack of regulation unlike the European Union and the United States, this situation results in the contamination of wastewater, effluents, rivers and drinking water. Emerging pollutants present a new global challenge about water quality with potentially important threats to human health and ecosystems. Therefore, the development of a coordinated, integrated, and collaborative strategy by countries in Latin America for the consumption, discharge, and disposition of these

compounds is fundamental to contribute to the reduction and prevention of negative impacts that emerging pollutants cause in the environment and public health.

Keywords: emerging pollutants, Latin America, nonylphenol

1.1 INTRODUCTION

In the last two centuries, the use of chemicals has contributed to the economic and social development worldwide. As a result, thousands of chemical mixtures (synthetic and natural) are now present in the environment and daily life. Global production of synthetic chemicals has increased exponentially, between 1903 and 2000 the annual chemical production increased from 1 million to 400 million tons and from 2002 to 2011 more than 50% of chemicals were classified as environmentally harmful and 70% with significant environmental impact. By 2017, 97.8 million tons were reported as hazardous to the environment and 91.8 million tons as hazardous to health (EUROSTAT, 2019; Gavrilescu et al., 2015; WWF, 2020). There are more than 80,000 chemicals that are used in manufacturing products to meet human needs such as, cleaning products, drugs (prescription and over the counter), cosmetics, fragrances, personal care products, among others. These products are used and disposed of globally in the environment after a manufacturing process as industrial waste (Naidu et al., 2016). Approximately 3,000 of these substances are used in drug manufacturing, besides the many intermediates used in their synthesis (Rodriguez-Narvaez et al., 2017) representing an annual consumption of 100,000-200,000 tons (Tijani et al., 2016). Most of these compounds can cause harmful effects on the environment (Postigo and Barceló, 2015; Rodriguez-Narvaez et al., 2017; Thomaidis N.S., 2012), some of the main effects reported are abnormal physiological processes, reproductive problems, increase in cancer incidences, bacteria resistance development and high toxicity (Gogoi et al., 2018). Each one of these chemicals has different action mechanisms so they cause a specific effect. If similar effects are shared they can make synergy and show effects at lower concentrations (Cassani and Gramatica, 2015). This makes identification and risk assessment a challenge for researchers (Bolong et al., 2009). Thus, it is important to adopt good practices and environmental policies to mitigate potential risks in human health and environment taking into consideration the evidence of damage without requiring absolute scientific certainty.

In general, water quality research focuses on nutrients, microbial contaminants, heavy metals and priority pollutants. Recently, there has been acknowledged a new type of pollutants ("emerging pollutants") that affect significantly the quality of water and causes potential public health and security problems (Bilal et al., 2019). However, due to its recent detection and low concentrations (µg L-1, ng L-1), there is a gap in knowledge about its occurrence, fate, behavior, risk assessment, and ecological and human effects. The 2016 upgrade of the NORMAN Network of the European Commission established 1036 substances as emerging pollutants (EPs). This list includes daily use products like surfactants, flame retardants, pharmaceuticals, personal care products, biocides, gasoline additives, polar pesticides, and their degradation products, and various proven or suspected endocrine-disrupting compounds (EDCs). The main source of EPs are municipal wastewaters (Deblonde et al., 2011) and conventional water treatment plants that are not designed to remove this kind of pollutants and their degradation resistant metabolites, so they are disposed of directly into water bodies (Van Zijl et al., 2017). The investigation of the composition of EPs and their removal during wastewater treatment is scarce, due to the lack of monitoring programs because of the non-existing specific regulations (Rasheed et al., 2019). The occurrence of EPs in wastewater, its behavior during treatment and the production of drinking water are then key areas that require immediate studies.

1.2 EMERGING POLLUTANTS AND ITS IMPORTANCE IN HUMAN AND ENVIRONMENTAL HEALTH.

EPs emissions have become an environmental global problem because of the limited regulations and worldwide consensus that establish legislations in this regard. The Environmental Protection Agency (EPA) defines the EPs as chemical compounds without regulations where its behavior, environmental and public health impacts are poorly understood (EPA, 2017). The high occurrence of EPs in the environment is not just attributable to chemical activities, drug manufacturers and climate change; there are other anthropogenic activities like mining, agriculture, domestic activities, and urbanization that may contribute to the increasing pollution index which prevents the availability of sustainable water sources (Phillips et al., 2010; Rasheed et al., 2019; Tijani et al., 2016). Also, the continuous and growing development of new products and manufacturing processes may cause an urgent need to determine if these compounds can be classified as EPs and/or EDCs. The EPA has defined them as exogenic agents that interfere with the synthesis, secretion, transport, binding or elimination of natural hormones in the human body that are responsible for the homeostasis, reproduction, development, and behavior (EPA, 2017). The endocrine system comprehends endocrine glands, hormones, and receptors. This regulates physiological activities such as reproduction. Therefore, the presence of these pollutants may affect the development of embryos, sexual differentiation, and metabolism (Flint et al., 2012). Some of the effects of EDCs are: altering the reproductive system, the sexually-dimorphic neuroendocrine system, affecting endogen levels of steroids, diabetes, cardiovascular problems, abnormal neuronal behavior and they are obesity-related (Arlos et al., 2018; Silva et al., 2018; Vilela et al., 2018). These xenobiotics are found in most products manufactured for personal use like plastic bottles, children toys, cosmetics, toothpaste, detergents, drugs (hormones, anti-inflammatory, anti-epileptic, statins, antidepressants, beta-blockers, antibiotics, contrast products, etc.), among others (Gavrilescu et al., 2015). These are mainly utilized in domestic, veterinary and in hospitals processes (Deblonde et al., 2011). The use of these chemicals has increased EDCs occurrence in wastewater, groundwater, surface water and drinking water in urban areas being the main pollution source wastewater from laboratories, hospitals and medical facilities (Gavrilescu et al., 2015). Nevertheless, there are other pollution sources as human and animal defecation (partially or completely metabolized), disposal of products without previous use (Gogoi et al., 2018; Poynton and Vulpe, 2009), domestic sewage, septic tanks, industrial effluents, urban wastewater, agricultural practices, showering, household cleaning, and recreational activities (Daghrir and Drogui, 2013). The table in supplementary material shows pollutants usually found in water bodies, which are: antibiotic, antacids, steroids, antidepressants, analgesics, anti-inflammatories, antipyretics, beta-blockers, lipid-lowering drugs, tranquilizers, stimulants, drugs (prescription and over the counter), phthalates, phenolic compounds (bisphenol A, nitrophenol, nonylphenol, alkylphenols, and chlorophenols), triclosan, ethinylestradiol, diethylstilbestrol, 17-β estradiol (Barrios-Estrada et al., 2018; Gogoi et al., 2018; Rivera-Utrilla et al., 2013; Stuart et al., 2012; Yan et al., 2014). Even though concentrations in the environment are at trace levels (µg L-1 or ng L-1) (Pal et al., 2012), these may generate adverse effects in water quality and represent an estrogenic risk for ecosystems and human health (Sirés and Brillas, 2012). Although some of these chemicals are hydrophilic, water-soluble and with a short life period (Fent, 2008), others can be persistent in the environment without degrading for years (Tijani et al., 2016). Water treatment plants currently employ just one type of technology and are not designed for the removal of EDCs presenting deficiencies in the removal. It is important the research and implementation of coupled systems to compensate for such shortcomings. It is an emerging issue in environmental sciences and engineering to accomplish effective removal processes of EDCs from wastewater before it is reintegrated into the environment (Ruhí et al., 2016). The fate and behavior of EDCs in the environment are complex and diverse so there is little information in this regard.

1.3 REGULATORY FRAMEWORKS FOR EMERGING CONTAMINANTS AND THE PROBLEM OF IMPLEMENTATION.

Outstanding in continuous monitoring measures and regulation of maximum permissible limits in environmental matrices are the United States and the European Union through their environmental regulatory agencies (Table 1.1). For example, the EPA created the Drinking Water Contaminant Candidate List which includes some drugs like carbamazepine, naproxen, sulfamethoxazole, ibuprofen, gemfibrozil, atenolol, diclofenac, erythromycin, and bezafibrate (EPA, 2018).

TABLE 1-1. COMPARISON OF REGULATIONS OF EMERGING POLLUTANTS IN THE UNITED STATES AND THE EUROPEAN UNION.

Region	Fundamental Law	Number of EPs	Supportive Agencies
United States	Safe Drinking Water Act	109	EPA FDA EDSTAC Global Water Research Coalition
European Union	Water Framework Directive	1036	EQSD Groundwater directive REACH Collaborative Project SOLUTIONS NORMAN

EPs: emerging pollutants, EPA: Environmental Protection Agency; FDA: Food and Drug Administration; EDSTAC: Endocrine Disruptor Screening and Testing Advisory Committee; EQSD: Directive on Environmental Quality Standards; REACH: Registration, Evaluation, Authorisation and Restriction of Chemicals; NORMAN: European Monitoring Network.

For the EU, it introduced a list of priority pollutants under the European Union Strategy for Endocrine Disrupters that includes 564 chemicals believed to have some form of disruptive activity (Stefanakis and J.A, 2015). Moreover, it constituted the EU Collaborative Project SOLUTIONS (Brack et al., 2015) and the European Monitoring Network NORMAN (www.norman-network.net) evaluating over 1036 EP. The mission of these investigation efforts is to improve evidence to make policies for identification, assessment, and prioritization of this compounds (von der Ohe et al., 2011).

Attempts to improve water and environment quality have resulted in the creation of regulation trying to reduce hazardous chemical use and production. However, even when significant advances have been accomplished, chemical pollution is still a substantial risk in at least half of the water bodies recently monitored in Europe (Malaj et al., 2014). The European Union (EU) reports more than 100,000 chemicals and between 30,000 to 70,000 are used in daily life (Loos et al., 2009). It is expected that an important, but unknown fraction of these chemicals finds its way into the environment and water systems with a considerable number of transformation products and byproducts that may create complex mixtures (Brack et al., 2015). There are regulatory frameworks to monitor and manage potential pollution sources of some priority pollutants in the aquatic environment, but EPs are not subject to the same regulations. There are no specific regulations for new compounds, byproducts, drugs, and personal care products so there are few or none precautions to assure the no disposal to the drainage (Bolong et al., 2009). Thus, wastewater is considered the main pollution source in the environment and surface water (Lapworth et al., 2012) as maximum concentration levels for disposal have not been established (Gogoi et al., 2018). The management and usage of these chemicals are challenging for the government because of the little information about impacts, fate and concentration levels of EPs in the environment; hence there are few global agreements on which EPs need to be observed (Rodriguez-Narvaez et al., 2017). There is a current and continuous urge to create and enforce new directives shortly because of the increasing and undefined risks that the exposure to EPs represent (Stefanakis and J.A, 2015). A monitoring strategy of EPs in water is essential to protect and improve water quality. The spaced and temporary variability of many EPs in the environment is a topic of growing interest from a regulatory and research perspective, so an increase of regulated compounds is expected soon. The main problem with EPs is the little information available and that most of them are still without regulation. Therefore, many countries are contributing to studying its occurrence and fate in the environment. **Table 1.2** shows current agencies and activities in the United States (US) and the European Union (EU) which have shown particular interest in establishing regulations for the monitoring and prevention of EPs.

TABLE 1-2. REGULATIONS OF NONYLPHENOL AND ITS ETHOXYLATES IN DIFFERENT REGIONS.

Region	Directive	Observations
European Union	2003/53/EC	Must not be commercialized or used as a substance in mixtures in quantities equal or higher than 0.1% in mass of NP or 1% in mass of NPE
	775/2004 (02/2076)	Prohibited for the elaboration of pesticides
	2013/39/EU	Listed as a priority pollutant and allows a maximum concentration in water of 2 µg L^{-1}
United States	EPA	Recommends concentrations of NP in fresh water to be lower than 6.6 µg L^{-1} and in saltwater lower than 1.7 µg L^{-1}
Canada	CEPA	Listed as toxic substance.
Signatory countries	Rotterdam Convention	Severely restricted compound

EPA: Environmental Protection Agency, CEPA: Canadian Act of Environmental Protection, NP: nonylphenol, NPE: nonylphenol ethoxylates

The regulation of pollutants in drinking water in the US began in 1962 with the Public Health Services Standards. The Safe Drinking Water Act of 1974 is the fundamental law governing drinking water security in the US. This law states that the EPA has to establish a maximum concentration level of some water pollutants including pesticides with endocrine disruption effects. However, endocrine disruption has not been named since 1995 with the amendments to the Safe Drinking Water Act (bill number S.1316) (Sen. Kempthorne, 1995) and the Food Quality Protection Act (bill number P.L. 104-170) (EPA, 2019) which states that chemicals and formulations have to be analyzed to determine if there is a potential endocrine activity before manufacture processes of food and/or drinking water. To accomplish these requirements, the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) was created. This agency provides recommendations about the conceptual framework, priorities, test methods, and monitoring, communication and outreach programs of approximately 87,000 chemicals. Even though there is a large legislative framework for EDs there is no maximum permissible limit for most of these compounds in surface and drinking water. Drugs that are disposed into the environment from manufacturers are regulated but they are not considered as an important pollution source (Bell et al., 2011); only when concentrations exceed 1 µg L-1 the Food and Drug Administration (FDA) requests ecological tests and drug assessment. Also, the EPA created the Drinking Water Contaminant Candidate List (EPA, 2018) which includes some drugs like carbamazepine, naproxen, sulfamethoxazole, ibuprofen, gemfibrozil, atenolol, diclofenac, erythromycin, and bezafibrate. These compounds have already been listed as drugs of primary importance by the Global Water Research Coalition (Coalition, 2008). More recently, the US organized an observation technique and recommended a management approach to decrease EDs without establishing maximum permissible concentrations in surface water, drinking water and/or wastewater (Commission, 2011).

One of the most important goals presented in the 7th Environmental Action Plan of the EU "Living well within the limits of our planet" (Union, 2013a) was to assure water quality and protect it from chemical pollution. The Water Framework Directive (WFD) and its directive daughters the 2008/105/EC Environmental Quality Standards Directive (EQSD) (Union, 2008) modified by the 2013/39/EU Directive which presents changes in the priorities substances list (Union, 2013b), and the Groundwater Directive 2006/118/EC (Union, 2006b) modified by the Directive 2014/80/EU (Union, 2014), are the most important legislation framework for the protection and sustainable use of water sources in the EU. The WFD published in 2000 included strategies and measures for pollution mitigation caused by chemical compounds listed as priority substances. Decision 2455/2001 (Commission, 2001) presented the priority substance list that included the chemicals considered as the most hazardous for the aquatic environment. The Directive on Environmental Quality Standards 2008/105 known as the Priority Substances Directive (Commission, 2008) formulated standards for 22 priority substances and 8 for other persistent, bioaccumulative and toxic pollutants. More recently, the WFD listed 45 priority compounds to observe in the aquatic environment and listed another 10 for monitoring (Decision 2015/495 published on 24 march of 2015) (Union, 2015). The legislation of water has triggered a series of monitoring activities; for example, the new European Regulation on Registration, Evaluation, Authorization, and Restriction of Chemicals (REACH, 2006) valid since 2007, seeks to achieve a better understanding of the pollutants in Europe by making the industries responsible for the assessment and risk management related with chemicals through the implementation of environmental risk assessment programs. The regulations of REACH dictate that compounds associated with irreversible and serious health impacts as cancer and of reproduction should be characterized as Substances of Very High Concern (SVHC) subjected to formal authorization and high control levels. Furthermore, since 2011 a special group (Endocrine Disrupters Expert Advisory Group) from the European Commission deals with scientific and identification issues of the EDs to support and provide information to the European Commission related to the assessment and identification of these pollutants (Munn and Goumenou, 2013). The EU introduced a priority pollutant list under the EU-Strategy for Endocrine Disrupters that includes 564 chemicals that are thought to have some kind of disruptive activity. From these, 147 compounds are persistent in the environment and 66 have been demonstrated to have endocrine activity including 60 chemicals that are possibly exposed to humans. Lately, another 52 chemicals have been considered as EDs and added to this list (Stefanakis and J.A, 2015). Moreover, a watch list (the first one published in 2015) was created (Article 8b of the EQS Directive 2008/105/EC) (Union, 2013b) to include emerging pollutants, however, monitoring information is limited. On a scientific field, new investigation activities led to founding the EU Collaborative Project SOLUTIONS (Brack et al., 2015) ant the European Monitoring Network NORMAN (www.norman-network.net). The mission of these investigation efforts is to improve evidence to make policies for identification, assessment, and prioritization of EPs (von der Ohe et al., 2011). However, the actual monitoring is not sufficient and inadequate in many of the Member States where not all the priority substances are monitored and the number of water bodies under supervision is limited (Solheim and Künitzer, 2012). Specific goals for Europe include identification, assessment, and management of hazardous compounds considering population increase and population age. Globally, the WFD is currently seen as a model for the implementation of activities to protect water quality and it has been receiving great attention by regulatory and scientific agencies worldwide. The WFD as a directive for all the European Union Members

States has successfully safeguarded the protection, restoration, and sustainability of water resources in the EU (Brack et al., 2017).

1.4 EMERGING POLLUTANTS IN LATIN AMERICA

EPs' concentration levels in the environment are different in each country and usually depend on the industrialization level, consumption patterns, regulations, population growth, water treatment plants efficiency, among others (Tijani et al., 2016). A population growth from 606 million to 780 million is expected by 2050 (Toshiko Kaneda, 2018) which may cause a biodiversity loss (Sanderson et al., 2009), water pollution (Jackson et al., 2001), land modification (Machovina et al., 2015) and an increase in energy demand (Valle, 2015). Research on EPs in Latin America is recent even though their concentration levels have increased significantly in the past 10 years (Bedoya-Rios et al., 2018; Botero-Coy et al., 2018; Hernandez et al., 2015). Usually, research has a scientific and academic approach instead of being a source for the creation and implementation of regulations and public policies (Peña-Guzmán et al., 2019). The most found and therefore most studied pollutants are pesticides, drugs, EDCs, plastics and nanomaterials that are generated by activities like agriculture, urban effluents, solid residues, paper mills and mining (Furley et al., 2018). Stumph et al (Stumpf et al., 1999) published one of the first studies where they found drugs and its metabolites in water bodies from Rio de Janeiro and discussed the lack of information of EPs in Latin America. Previous studies in Latin America have identified 197 emerging pollutants in the environment, being Mexico and Ecuador the countries that have carried out more studies in this regard, usually finding in wastewater compounds like ibuprofen, carbamazepine, trimethoprim, sulfamethoxazole, caffeine and naproxen (Peña-Guzmán et al., 2019). Analytical methods and limits of detection and quantification (LOD/LOQ) used in Latin America are similar to those employed by other countries. However, there is an important difference between concentrations found in Latin American compared to concentrations found in developed countries (**Table 1.1**). Latin American countries with less protected areas, present a larger population and major biodiversity loss. However, to the date, there is no estimation of the relative impact of the pollution in biodiversity (Rodriguez-Jorquera et al., 2016). The aquatic environment may be the most threatened habitat because the wetlands sustain biodiversity and provide essential ecosystem services (Zedler and

Kercher, 2005). Therefore, there are initiatives like the project RLA/7/019 “Elaboration of indicators to determine the effects of pesticides, heavy metals, and emerging pollutants in continental aquatic ecosystems important for agriculture and agro-industry” in which some Latin American countries (Brazil, Chile, Cuba, Costa Rica, Guatemala, Ecuador, Nicaragua, Panama, Paraguay y Uruguay) take part. The main objective is to improve life quality, food security and agricultural resources in Latin America and the Caribbean through strong management of aquatic resources. This project promotes communication through pamphlets, videos, and documents encouraging the use of risk maps(Atómica, 2019). These projects are in response to the creation of the 2030 goals for sustainable development established by the ONU (www.un.org/sustainabledevelopment/sustainable-development-goals). The projects’ goals include 17 goals for sustainable development and 169 objectives that seek to complete the previous Millenium Development Goals of 2000 (www.unmilleniumproject.org/goals). Some of these goals depend on the implementation of management environmental decisions. The identification of specific programs of investigation to support the environmental management goals occur as a response of the population needs and they are modified according to priorities and resource availability over time. Although this kind of initiative exists, the lack of regulations in Latin America allows an accelerated and increased discharge of EPs representing an important risk in environmental and public health impacts (Qiu et al., 2016; Silva et al., 2018).

1.5 CASE STUDY OF UNDER-STUDIED EMERGING POLLUTANTS IN LATIN AMERICA: NONYLPHENOL

Nonylphenol ethoxylated (NPE) is a non-ionic surfactant usually employed as a surfactant in domestic and industrial detergent formulations (Quiroga et al., 2019; Vazquez-Duhalt et al., 2005). The use of NPE in detergents represents 80% of its demand because of the low cost and the great detergent capacity (Araujo et al., 2018). Another uses include herbicides, pesticides and in production of paper, textiles, plastics, rubbers and paints (Ferrara et al., 2011; Perron and Juneau, 2011). The high industrial demand and its use in anthropogenic activities have increased its occurrence as a pollutant in sewage sludges and landfills mainly because of waste discharges, water treatment plants and accidental spills (Qiu et al., 2016; Silva et al., 2018). In environmental conditions, NPE is degraded by microorganisms or ultraviolet light transforming them into nonylphenol (NP) including mainly the 4-nonylphenol (4-NP) (**Figure 1.1**) (Cheng et al., 2017). It has been reported that this metabolite is more resistant to degradation, possesses endocrine disruptive activity and is highly toxic to aquatic and terrestrial organisms because of its ability to mimic the feminine hormone 17β-estradiol (Forte et al., 2016). Removal percentages of NP in water treatment plants vary from 9 to 94% depending on the process used. For example, a previous study showed only 9% of biodegradability after 56 days in water and 4.2% after 28 days in sediments (Vazquez-Duhalt et al., 2005). The removal efficiency is affected because the conventional treatment plants are not designed to process this kind of pollutants (Ruhí et al., 2016) and because of the chemical structure stability of these compounds (Gavrilescu et al 2014). Efficient degradation of endocrine disruptors may need coupled systems to compensate deficiencies of using just one technology for the removal of these complex compounds in water (Rodriguez-Narvaez et al 2017). Novel techniques like laccase-assisted degradation of EPs and EDCs have received particular interest because of its uniqueness and applicability (Barrios-Estrada et al 2018). Previous studies have efficiently treated environmental polluted matrixes with nonylphenol using laccase-assisted degradation (Tanaka et al 2003; Catapanea et al 2013; Tsutsumi et al 2001). Thus, the development of new technologies for treating EPs in wastewater treatment plants is necessary for environmental monitoring and as a support on policies to control concentrations of EPs in wastewater. Also, the NP can transfer between water and air because of its semi-volatile property so it can be transported to aquatic and terrestrial ecosystems by wet deposition (rain or snow) (Chen et al., 2013). Even though the concentration of NP can decrease because of the effect of the sun over surface water, there is evidence that NP in sediments has an average life span of over 60 years which represents a constant exposure to all organisms (Soares et al., 2008).

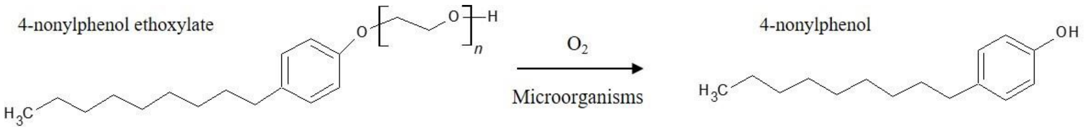

FIGURE 1-1. DEGRADATION OF NONYLPHENOL ETHOXYLATE IN THE ENVIRONMENT.

1.5.1 EFFECTS ON ECOLOGICAL HEALTH BY NP EXPOSURE AND IMPLICATIONS IN LATIN AMERICA

The main disruptive endocrine activity of the NP is the ability to mimic the feminine hormone 17β-estradiol. However, this is not the only mechanism that the NP uses to disturb the endocrine functions of organisms. Other examples include change of sex, raise of hermaphroditism, decrease of testosterone elimination, fecundity reduction, mutations, deformities, increase in mortality, gonadal development inhibition, low testicular mass, ovaries development inhibition and fertility reduction (Buñay et al., 2017; Forte et al., 2016; Patino-Garcia et al., 2018; Uguz et al., 2015). Previous studies in fish have demonstrated adverse effects by NP exposure. It has been shown a gonadosomatic index decrease in a red seabream and black rockfish associated to the exposure of 50 µg L-1 of 4-NP (Saravanan et al., 2019); Japanese quails exposed to 4-NP presented a decrease in male spermatogenesis and pathologic damage in male gonads (Cheng et al., 2017); the immune system of rainbow trouts (Oncorhynchus mykiss) was affected after NP exposure with concentrations considered as safe for aquatic species (1 µg L-1) (Hébert et al., 2009); a kind of fish with hermaphrodite tendency (Acanthopagarus latus) and the more marketed for its consumption in Iran exposed to 4-NP showed alterations in steroids and thyroidal levels (Naderi et al., 2014). Organisms without endocrine systems are not immune to the effects of NP. Some studies have evidenced that exposure to NP may cause undesired morphological, physiological and structural effects in different types of plants. This may result in exposure to humans and/or affect the natural defense system causing growth disorders (de Bruin et al., 2019).

Studies about endocrine disruptive adverse effects of NP exposure focus principally on reproduction functions, immunity, and its potential as a carcinogen agent. However, other potential adverse effects have not been examined extensively. Cytotoxicity studies in different types of cell lines by NP exposure have been made to increase information in this regard. It has been found mainly that NP exposure causes apoptosis provoking morphological distinctive changes in different types of cells like thymocytes, PC12 cells, sperm cells and testicular Sertoli cells (Kim et al., 2007), as well as prostatic cell lines and ovaries under a depending activation process ADAM17 (Urriola-Munoz et al., 2018). Cytotoxicity effects in Sertoli cells have been explored and it has been found that the exposure to 20-30 μM of NP for 24 hr causes a decrease in cellular viability provoking cellular death via apoptosis and autophagy (Duan et al., 2016). Neural mother cells have been studied after being exposed to 4-NP by 24 hr finding that cellular growth is inhibited in a concentration dependent manner and inducing apoptosis (Kudo et al., 2004). Oral exposure of 4-NP in rats causes a significant reduction in the capability of forming colonies and duplicating mother mesenquimals mother cells population which prevents bone regeneration and originates potential risks of acquiring bone diseases like osteoporosis (Abnosi and Shojafar 2014). NP exposure in the skin needs to be considered as well because previous studies have already reported NP absorption in different species. NP exposure in a type of fish (Salmo salar) increased the hepatic estrogenic receptors detected in skin (Arukwe and Røe, 2008), pathological alterations in skin of amphibians that can easily interfere in physiological functions have been reported (Brunelli, 2018) and there are available reports stating that the exposure to low concentrations of NP may increase cases of atopic dermatitis in mammals (Sadakane et al., 2014). Seven different compounds of alkylphenol have been found in adipose subcutaneous tissue in humans, being the NP the most common (Ferrara et al., 2011). Even though there are few studies in this regard, it has already been found NP accumulation in adipose tissue in humans and cadavers with concentrations of 57 and 37 ng g-1 respectively (Lopez-Espinosa et al., 2009).

Effects after NP exposure like the alteration of cellular functions as the suppression of natural killers' human cells (first line of defense against tumoral cells and virally infected) have been previously reported. This leaves the individual more susceptible to develop cancer or acquiring a viral infection (Udoji et al., 2010). Even when there is proof that NP can be removed effectively from the organism (from 1 to 4 weeks) the continuous exposure may affect the elimination efficiency (Vazquez-Duhalt et al., 2005). There is scarce evidence of the effects in humans but NP exposure so studies in this regard are needed promptly.

1.5.2 HUMAN RISK TO NP AND IMPLICATIONS IN LATIN AMERICA

NP exposure in humans is mainly through the consumption of polluted water and foods (Careghini et al., 2015). Due to its high stability and solubility in lipids, NP may be accumulated in fish internal organs reaching concentrations of 10 to 100 times higher than concentrations found in the environment (Kim et al., 2007). This originates an important concern because it could be easily transmitted to humans through the food chain (Chang et al., 2019). Likewise, variable concentrations of NP found in multiple foods (independent of the fatty content and packing materials) imply that NP is introduced in different production stages. This may be because of its use as a non-ionic surfactant and/or emulsifier in disinfectants, cleaning products and pesticides (Kawamura et al., 2017; Mao et al., 2012). Therefore, it is important to detect its presence in seafood, beverages, egg, vegetables, fruit, meat, rice, commercial milk, among others (Sise and Uguz, 2017). Variable concentrations of NP have been reported previously; for example, in packaged food concentrations from 0.1 to 19.4 µg kg-1 (7.5 µg NP per day) (Guenther et al., 2002), in commercially available fruits, cereals, and vegetables from 10 to 71 µg kg-1 (Gyllenhammar et al., 2012), in packaged fruits and vegetables from 14.5 to 48 µg kg-1 (Cacho et al., 2012), in local fruits and vegetables markets from <0.3 hasta 11.0 µg kg-1, in lettuce and cabbage leaves from 1.18 to 6.95 µg kg-1 and roots from 339.2 to 926.9 µg kg-1 (Dodgen et al., 2013) and stew from 4.32 to 167 µg kg-1 (Hao et al., 2018). Concentrations in meat and seafood have also been reported; for example, concentrations in meat and seafood from supermarkets from <0.05 to 55.98 µg kg-1, in edible marine species from 5 to 1220 µg kg-1 (Ferrara et al., 2011), aquatic organisms from 122 to 2380 µg kg-1 (Diehl et al., 2012) and up to 918 µg kg-1 in grains and can food (animal and seafood origin) (Chang et al., 2019). No studies about nonylphenol in foods in Latin America were found. Thus, exposure levels through food are difficult to establish.

The main exposure pathway in infants is through breast milk intake. Previous investigations have found up to 32 ng mL-1 of NP in breast milk in Italian women (Ademollo et al., 2008), from 17 to 11.6 ng mL-1 in women from Taiwan (Lin et al., 2009) and up to 47 ng mL-1 in women from Turkey (Sise and Uguz, 2017). The intake of NP of children exclusively fed with breast milk in Germany is approximately 1.4 µg per day (Guenther et al., 2002) and children with a mixed diet are approximately from 0.23 to 0.65 µg kg-1 (Raecker et al., 2011). A child´s diet includes also cow milk so it is important to consider it. It has been reported concentrations of NP in commercial cow milk from 2.9 to 8.9 ng g-1 in Taiwan (Lin et al., 2009), 0.3 ng g-1 in Germany (Guenther et al., 2002) and 1.0 ng g-1 in Japan (Otaka et al., 2003). The information about concentrations of NP in food is limited, without mentioning the little information available on the potential risk after its consumption.

1.5.3 REGULATION FOR THE USE OF NONYLPHENOL AND THEIR ETHOXYLATES

Strategies and regulations have been developed to deal urgently with EDCs, among them NP (Table 3). For example: (i) The EU restricted its use with the Directive 2003/53/EC which establishes that it must not be commercialized or used as a substance in mixtures in quantities equal or higher than 0.1% in mass of NP or 1% in mass of NPE (Union, 2003). Moreover, the Directive 775/2004 (02/2076) prohibited its use for the elaboration of pesticides (Union, 2006a), (ii) The Water Framework Directive of the European Union with the Directive 2013/39/EU has listed NP as a priority pollutant and allows a maximum concentration in water of 2 µg L-1 (Union, 2013b), (iii) the Rotterdam Convention (2012) classified it as a prohibited chemical or rigorously restricted in the EU and Switzerland, (iv) the governing body of the Convention for the Protection of the Marine Environment of the Baltic Sea classified the NP as dangerous according to the action plan for the conservation of the Baltic Sea (UNEP, 2015), (v) EPA recommends concentrations of NP in freshwater to be lower than 6.6 µg/L and in saltwater lower than 1.7 µg/L (EPA, 2005), (vi) The Canadian Act of Environmental Protection added this substance to the 1999 list of toxic substances (Canada, 1999).

These restrictions summed with the impacts previously discussed are forcing the academic and industrial community to develop strategies to substitute the NPE to minimize health and environmental risks. Currently, Latin America does not rely on any regulation in this regard, so it is necessary to increase the number of environmental and health authorities that create and implement regulations and restrictions to reduce environmental impacts that may represent a risk to public health. (Peña-Guzmán et al., 2019).

1.5.4 ENVIRONMENTAL LEVELS OF NP IN THE WATER OF LATIN AMERICA COMPARED TO OTHER REGIONS

Even though the presence of EPs like the NP is known worldwide there is a gap of knowledge about its distribution in some geographic areas like Latin America (Llorca et al., 2017). A compilation of studies of NP concentrations in different water bodies worldwide is shown in **Table 1.3**.

TABLE 1-3. CONCENTRATIONS OF NP IN WATER SAMPLES WORLDWIDE.

Country	Sample	n	Min	Median	Max	Reference
Mexico	Surface water	8	830	3 050	12 610	(Vargas-Berrones et al., 2020)
Mexico	Wastewater	12	<LOD	3 790	12 200	(Vargas-Berrones et al., 2020)
Mexico	Drinking water	5	<LOD	2 480	6 080	(Vargas-Berrones et al., 2020)
Argentina	Surface water	14	1 730 000	2 020 000	2 390 000	(Babay et al., 2008)
Argentina	Wastewater	6	2 390 000	2 550 000	2 680 000	(Babay et al., 2008)
Brazil	Surface water	5	ND	ND	1240	(Jardim et al., 2012)
Brazil	Drinking water	5	ND	<LOQ	<LOQ	(Jardim et al., 2012)
Brazil	Surface water	2	<LOD	<LOD	<LOD	(Raimundo, 2011)
Mexico	Surface water	1	89	-	655	(Félix–Cañedo et al, 2013)
Mexico	Drinking water source	1	1	-	47	(Félix–Cañedo et al., 2013)
Mexico	Surface water		930	-	7 600	(Belmont et al, 2006)
Mexico	Wastewater		750	-	13 020	(Belmont et al., 2006)
Colombia	Drinking water source	5	ND	ND	ND	(Martinez & Peñuela, 2013)
France	Surface water	12	6	58	550	(Fenet et al., 2003)
China	Surface water	15	100	1 300	7 300	(Shao et al, 2005)

TABLE 1-4. CONCENTRATIONS OF NP IN WATER SAMPLES WORLDWIDE.

China	Drinking water	15	10	50	2 700	(Shao et al., 2005)
Spain	Surface water	6	500	2 000	36 000	(Martin et al, 2014)
Spain	Wastewater	4	600	142 000	289 000	(Martín et al., 2014)
Italy	Wastewater	8	370	515	700	(Loos et al, 2007)
Italy	Drinking water	35	<7.7	14.9	84	(Maggioni et al, 2013)
Belgium	Wastewater	10	250	745	2 500	(Loos et al., 2007)
Korea	Surface water	18	10	900	41 300	(Li et al, 2004)
Iran	Wastewater	9	420	807	2 120	(Bina et al, 2018)
South Africa	Surface water	7	380	1655	2610	(Sibali, 2010)
United States	Wastewater (influent)	8	265 000	745 000	745 000	(Loyo-Rosales et al, 2007)
United States	Wastewater (effluent)	8	42	5970	8420	(Loyo-Rosales et al., 2007)

*Units: ng L^{-1}, LOD: limit of detection, LOQ: limit of quantification, ND: not detected.

Currently, water bodies with concentrations lower than 1 µg L-1 are considered as poorly contaminated, with concentrations between 1-10 µg L-1 are considered as contaminated and with concentrations above 10 µg L-1 are considered as highly contaminated. With the above, and considering that a woman can produce from 10 to 100 µg L-1 of estrogens a day, the concentrations reported in Latin America are alarming (Soares et al., 2008). The lack of information in countries of Latin America like Cuba, El Salvador, Haiti, Honduras, Nicaragua, Panamá, Paraguay, Peru y Republica Dominicana can be attributed to the economy mainly because of the high cost of mediating political environmental interests (Peña-Guzmán et al., 2019). It is important to implement a strategy that includes continuous monitoring and the implementation of regulations for the environmental impact assessment and the possible public health risks in Latin America.

1.6 TECHNOLOGICAL DEVELOPMENT FOR SUBSTITUTION OF NPE IN DETERGENTS

NPE is substituted usually by ethoxylated alcohols; less effective surfactants but safer for the environment because of its fast biodegradation. However, some studies suggest that biodegradation byproducts have low solubility and are absorbed in residual sludges (Soares et al., 2008). On the other hand, natural surfactants may also substitute alkylphenol ethoxylates; for example, alkyl polyglucosides (APGs) are nonionic surfactants produced by removable raw materials like corn, potatoes, wheat and coconut oil (Pantelic and Cuckovic, 2014). This compound has advantages over other surfactants: appropriate critical micellar concentration, ability to form microemulsions, proper foaming power, stable at different pH values, biodegradable, low aquatic toxicity and favorable dermatological properties (Holmberg, 2001; Jurado et al., 2012; Pantelic and Cuckovic, 2014; Qin et al., 2006). Due to its natural origin and the excellent physical and environmental properties, the APGs may be used in domestic and industrial applications (Rios et al., 2016). Even though this compound has some advantages it is still a challenge to persuade companies to replace conventional surfactants because of the higher costs of natural surfactants (up to 50 times higher than synthetic surfactants) (Deleu and Paquot, 2004).

1.7 CONCLUSIONS

Emerging pollutants present a new global challenge about water quality with potentially important threats to human health and ecosystems. The importance of this issue is such that the United Nations included it in the Objectives of Sustainable Development as Goal No. 6 (Ensure access to water and sanitation for all). There is a lack of information about EPs occurrence in some regions of the world, significantly in developing countries such as the Latin American countries. This review identified that EPs occurrence is linked with the lack of legislation, high analysis costs in environmental matrixes and little information about probable effects. Nevertheless, it is necessary to adopt good practices and environmental policies to mitigate potential risks in human and ecological health based on the approach of precautionary principles, based on evidence of possible harm without requiring absolute scientific certainty. The example of NP showed in this review, highlights the lack of regulation in Latin America regarding EPs, which results in pollution of wastewaters, effluents, rivers and drinking water. The development of a coordinated, integrated and collaborative strategy by countries in Latin America for the consumption, discharge, and disposition of these compounds is fundamental. This could contribute to the reduction and prevention of negative impacts that EPs cause in the environment and public health. It is imperative to determine potential effects, occurrence and concentration levels to improve regulations of these pollutants soon.

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2. RAPID ANALYSIS OF 4-NONYLPHENOL BY SOLID PHASE MICROEXTRACTION IN WATER SAMPLES.

Talanta 209 (2020) 120546

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Rapid analysis of 4-nonylphenol by solid phase microextraction in water samples

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ABSTRACT

Alquilphenols are considered to be endocrine disruptors and are mainly found as 4-nonylphenol (4-NP) in ecosystems. A chromatographic analytical method was developed using solid phase microextraction (SPME) combined with gas chromatography-mass spectrometry (GC-MS) for the extraction and quantification of 4-NP in sources of water in Mexico to establish environmental concentrations. A derivatization process with N-Methyl-bis(trifluoroacetamide) (MBTFA) was carried out in order to increase sensitivity in the method. The method was validated with a correlation coefficient above 0.99, and a limit of detection and quantification of 0.01 µg L^{-1} and 0.15 µg L^{-1} respectively, which were determined by a linear curve at low 4-NP concentrations. After demonstrating the feasibility of the method, an analysis in water samples was performed. Eighty-three percent of samples had detectable concentrations of 4-NP with a maximum concentration of 12.61 µg L^{-1}, 12.2 µg L^{-1} and 6.08 µg L^{-1} in recreational water, wastewater discharges and drinking water respectively. Sixty-five percent of the samples presented concentrations above the limit established by the European Union (2 µg L^{-1}) and 17% above the limit by the Environmental Protection Agency (6.6 µg L^{-1}). Although the number of samples is not representative for the assessment of the real-world scenario, our data allows presents a general overview of the exposure levels and possible environmental and health risks. Continuous monitoring and regulation of this pollutant in Mexico is important in order to prevent exposure and thus, ecological and adverse health effects.

Keywords: 4-Nonylphenol; Mexico; water; solid phase microextraction; gas chromatography-mass spectrometry.

HIGHLIGHTS

♦ A low-cost methodology to quantify 4-nonylphenol in water samples has been developed.

♦ It was demonstrated that the use of MBTFA as a derivatizing agent is adequate for the quantification of 4-NP offering high sensitivity to the method.

♦ The effectiveness of this analytical technique was assessed by monitoring 4-NP in different scenarios and sources of water in Mexico.

2.1 INTRODUCTION

Alquilphenol ethoxylates (APE) are non ionic surfactants widely used in detergents, plastic additives, emulsifiers, pesticides, among others. The APE most used is the nonylphenol etoxylate (NPE), its use represents up to 80% of the total production (Araujo et al, 2018). The main characteristic of this compound is that is capable of generating micelles in solution. The anphyfilic structure of the surfactant molecule, which has a polar (hydrophilic) and a non polar (hydrophobic) end, is the cause for this activity. These chemicals are disposed directly into municipal and industrial wastewater discharges. In environmental conditions, NPE are degraded by microorganisms or ultraviolet light by transforming them in Nonylphenol (NP) including the 4-Nonylphenol (4-NP) (La Guardia et al, 2001).

4-NP is one of the most found organic pollutant in wastewater, effluents, rivers, drinking water, sediments and soil (Belmont et al, 2006; Chen, 2013; Dong et al, 2015; Jie et al, 2017; Van Zijl, 2017). Several authors have reported that 4-NP possess endocrine disruption activity and is highly toxic to acuatic and terrestrial organisms because of its ability to mimetize the female hormone 17β-estradiol (McCormick et al, 2005). Previous studies have shown a decrease in the gonadosomatic index in red seabream and black rockfish associated with the exposure of 50 µg L^{-1} of 4-NP (Saravanan et al, 2019). Also, japanese quails exposed to 4-NP presented a decrease in male spermatogenesis as well as pathologic injuries in male gonads [10]. The Water Framework Directive of the European Union has listed NP as a priotary pollutant and allows a maximum concentration in water of 2 µg L^{-1} (Cheng et al, 2017), while the Water Quality Criteria for the acuatic organisms protection of the Environmental Protection Agency (EPA) establishes 6.6 µg L^{-1} as the maximum concentration permitted (EPA, 2005). Regulations in Mexico and Latin America that restrict the levels of NP in water are null. In addition, information on the presence of this pollutant in surface and treated waters is scarce. A study in Texcoco river, which is a wastewater receptor of Mexico City, presented levels of 4-NP from 0.93 to 7.3 µg L^{-1} (Belmont et al, 2006). In addition, agricultural irrigation water used in Tula Valley in Hidalgo presented concentrations from 1.98 up to 7.3 µg L^{-1}. Moreover, studies in drinking water of the Cutzamala system, which supplies 60% of water consumption in Mexico City, has registered concentrations of 0.089 to 0.655 µg L^{-1} (Felix-Canedo et al, 2013). Therefore, it is important to establish monitoring strategies in water in order to contribute to the generation of regulations in this respect, due to the toxicity associated to 4-NP and the wide use of NPE in Mexico.

An appropriate analytical method is necessary to quantificate 4-NP in different sources of water to be able to draw conclusions about the disposition and the possible threat and negative impacts that its use represent (Gatidou et al, 2007). Some of the most used methods for the analysis of alquilphenol (AP) are liquid chromatography (LC), mass spectrometry (MS) or tandem mass spectrometry (MS/MS) (Azzouz and Ballesteros, 2014). NP extraction in water samples is commonly performed by liquid-liquid extraction (LLE) (Jiang et al, 2014; Jiang et al, 2017) nevertheless, its popularity has fallen because of the high consumption of time and solvents. To this extent, solid phase extraction (SPE) is primarly used for the extraction and pre-concentration of NP from several environmental matrices (Chen et al, 2016; Fabregat-Cabello et al, 2013; Pernica et al, 2015). Although, compared to LLE, SPE reduces extraction time and solvent consumption, new analytical techniques that minimize costs, extraction time, organic solvents, quantity of sample and with high sensitivity, are being explored (Shih et al, 2015; Gong et al, 2015). Pawlisyn (2013) (Pawliszyn, 2013) reported for the first time solid phase microextraction (SPME) as a simple, rapid and non-solvent needed extraction method. SPME employs an automatic device which contains a thin polymeric coating that is adapted to a microsyringe. There are two types of SPME: i) direct immersion (DI) and ii) headspace (HS). DI is mainly used to extract NP due to its non-volatile nature; however, HS has also been used combined with a derivatization process (Chunmei and Snyder, 2018). Derivative formation increases volatility, decreases polarity in organic compounds and improves the thermal stability of analites. This causes a lower detection limit and/or better selectivity (Farajzadeh et al, 2014). The aim of this study was to develop and validate an analytical method for the quantification of 4-NP in water samples using SPME with GC-MS with a previous derivatization process. Subsequently, an evaluation of 4-NP in different sources of water in Mexico was performed to demonstrate the effectiveness of the method as a monitoring technique of this contaminant in multiple scenarios.

2.2 METHODS

2.2.1 MATERIALS AND REAGENTS

Stock standard solution of 4-NP (1000 µg L^{-1}) was prepared in acetone and stored in the dark at -40°C until its further analysis. Standard working solution was prepared right before its use.

A 0.1 M hydrochloric acid (HCl) (JT Baker, Edo. Mexico) solution, sodium chloride (NaCl) (JT Baker, Edo. Mexico), Milli-Q deionized water (18.3 MΩ, Millipore) and N-Methyl-bis(trifluoroacetamide) (MBTFA) ≥97.0% GC (Sigma Aldrich, Edo. Mexico) were used for derivatization process. Supelco supplied (Edo. Mexico) SPME fibers PDMS (100µm polydimethylsiloxane); PA (85 µm polyacrylate) and DVB/CAR/PDMS (50/30 µm stableflex divinylbenzene/carboxen/ polydimethylsiloxane) were tested for its performance. All three fibers were conditioned before use as manufacturer´s instructions.

Samples and calibration curves were analyzed in a gas chromatograph (GC) (Agilent 6890) coupled to a mass spectrometry detector (MS) (Agilent 5975) in electron impact ionization mode (EI). The injection port was operated in splitless mode with a 0.75 mm liner without glass wool. Injection port temperature was 230 °C; helium used as carrier gas at a pressure of 36 psi with a constant flow of 1 mL min^{-1}. The chromatographic separation was carried through a HP 5MS (60 m x 0.25 mm x 0.25 µm) column (Agilent). Setting of the oven was as follows: 90°C (2 min), 180°C (30°C min^{-1}), 200°C (1°C min^{-1}), 230°C (30°C min^{-1}) and held for 5 min with a run time of 31 min. The tune parameters were: emission: 34.6; energy: 69.9; repeller: 26.6 and, EMVolts: 1341. SCAN mode (50-500 m/z) was employed in order to identify the compound, identification and quantification ions were selected for SIM mode. The identified fragment ions were 203/316 m/z. Results were obtained and processed using Chemstation Software (Agilent).

2.2.2 ANALYTICAL PROCEDURE

The 4-NP quantification was performed with slight modifications of the methodology described by Basher & Lee (2004) (Basheer and Lee, 2004). For the calibration curves one milliliter of Milli-Q water was fortified with 4-NP using independent concentrations ranging from 0.5 µg L^{-1} up to 50 µg L^{-1} in amber glass vials. Sodium chloride (NaCl, 3%) and 0.1 M HCl (20 µL) were used to enhance ionic strength and to adjust pH, respectively. Standard working solutions were prepared right before its use. After sample extraction a derivatization process was carried out in order to improve volatility and sensitivity. This process consists on exposing the fiber to the headspace above a solution of acetone (1 mL) with MBTFA (100 µl) at 60°C for 10 min magnetically stirred at 600 rpm.

2.2.3 ESTABLISHING OPTIMAL CONDITIONS

Some parameters related with the adsorption of the analytes in the fiber were optimized to enhance the developed method: i) fiber coating, ii) temperature, iii) performance time and iv) ionic strength. For the optimization of the SPME procedure 1 mL of Milli-Q water was prepared fortifying with 1000 µg L^{-1} of 4-NP. NaCl and HCl (0.1 M) were added to adjust pH in an amber glass vial with magnetic agitation. Afterwards, the fiber was exposed to the headspace above de solution. Three types of fibers were evaluated for nonpolar, semipolar and polar compounds (PDMS, 100 µm, DVB/CAR/PDMS 50/30 µm, PA 85 µm). Assays were performed by triplicate to evaluate temperature (60, 80, 100°C), extraction time (5, 10, 20, 30, 40 and 60 min) and ionic strength (0, 3, 7%); averages are reported.

2.2.4 STATISTICAL ANALYSIS

A multivariate two-way ANOVA analysis was carried out to establish significant differences between the three fibers used considering two parameters: i) performance time (5, 10, 20, 30, 40 and 60 min) and ii) temperature (60, 80, 100°C). Determinations were performed by triplicate at a concentration of 1000 µg L^{-1} of 4-NP. The data distribution was evaluated using the Shapiro-Wilk test. Descriptive statistics parameters were calculated (mean, standard deviation, median, minimum and maximum). Differences between 4-NP concentrations reported in each scenario were established by the Kruskal Wallis test with a post hoc Dunn analysis using the statistic software GraphPad Prism 5.0 ®.

2.2.5 ANALYTICAL TECHNIQUE VALIDATION

The validation of the method was performed based on the Guide for the Validation of Analytical Methods for the Determination of Organic Compounds at Trace Levels AOAC/FAO/IAEA/IUPAC (AOAC/FAO/IAEA/IUPAC) considering the following parameters, limit of detection (LOD), limit of quantification (LOQ), linearity (r), sensitivity, percentage of recovery and precision (repeteability and reproducibility), evaluating different concentrations by triplicate on the same day, and by duplicate five different days. LOD and LOQ were calculated using results obtained from five calibration curves determined in a concentration range of 0.5 to 50 µg L^{-1}. The linearity expressed by the correlation coefficient (r) and the sensitivity determined by the slope of the working range curve (LOQ-50 µg L^{-1}) were obtained from the average of five curves obtained during three days.

2.2.6 MONITORING OF 4-NP IN WATER SOURCES

A total of 29 samples were collected, from which 10 were of recreational use, 14 of wastewater discharges and 5 of drinking water. Samples were collected in one-liter plastic recipients previously rinsed with Mili-Q water, none detergent was used in order to prevent contamination. After sampling, samples were stored in the dark at -20°C until its further analysis. The sampling sites were positioned using a Garmin GPS in UTM coordenates. Samples were collected mainly from the city of San Luis Potosí, México, in which the last decade its tourism has increased with the cooperation of national commissions and that has a growing industrial zone where metal processing, auto part manufacturing, froundries and chemical industries are the principal performed economic activities (Leon-Martinez et al, 2018). A few samples were taken from *cenotes* (natural pits, or sinkholes, resulting from the collapse of limestone bedrock that exposes groundwater underneath) located in Merida, Yucatán, Mexico. The lack of an integral sewerage and drainage networks makes this city highly vulnerable to antropogenic contamination (mainly agricultural) due to the final disposition of wastewaters directly in the aquifer. Urban wastewaters are frequently disposed with particular septic tanks followed by inflitration wells causing pollution in the groundwater. The drinking water samples were collected randomly from water jugs of 5 different brands.

2.3 RESULTS AND DISCUSSION

2.3.1 METHOD OPTIMIZATION

Different parameters affect sensitivity in SPME such as the fiber coating, extraction mode, and variables like temperature, stirring, ionic strength and extraction time (Pawliszyn, 2013). Our method considered the fiber type, extraction temperature, extraction time and ionic strength for the extraction of 4-NP. Head space (HS) extraction mode was selected for the extraction of 4-NP due to the fact that unlike DI extraction mode, the HS prevents fiber damage that could be caused for direct contact with high molecular weight compounds and other non-volatile substances in the sample. Additionally, pH can be adjusted without damaging the fiber (Pawliszyn, 2013). Even though, DI appears to be more addecuate for 4-NP extraction, HS extraction mode has been used successfully before in complex matrices (Sanchez-Avila,2017) and has been assessed with a previous derivatization process in environmental samples (Chunmei and Snyder, 2018). SPME is based on the equilibrium between the quantity of analite extracted by the fiber and the sample concentration, so the selection of the fiber coating is a key to determine the sensitivity, selectivity and repeteability of an analytical method (Alam et al, 2015). To this extent, three differents types of fibers were considered in this study: PDMS 100 µm (nonpolar compounds), DVB/CAR/PDMS 50/30 µm (semipolar compounds) and PA 85 µm (polar compounds).

The best conditions in the present study were achieved with a concentration of 3% NaCl. A greater chromatographic response was obtained. (**Figure 2.1**). Salt adition to samples has been frequently used in HS-SPME to moderately improve the extraction efficiency (Gong et al, 2015).

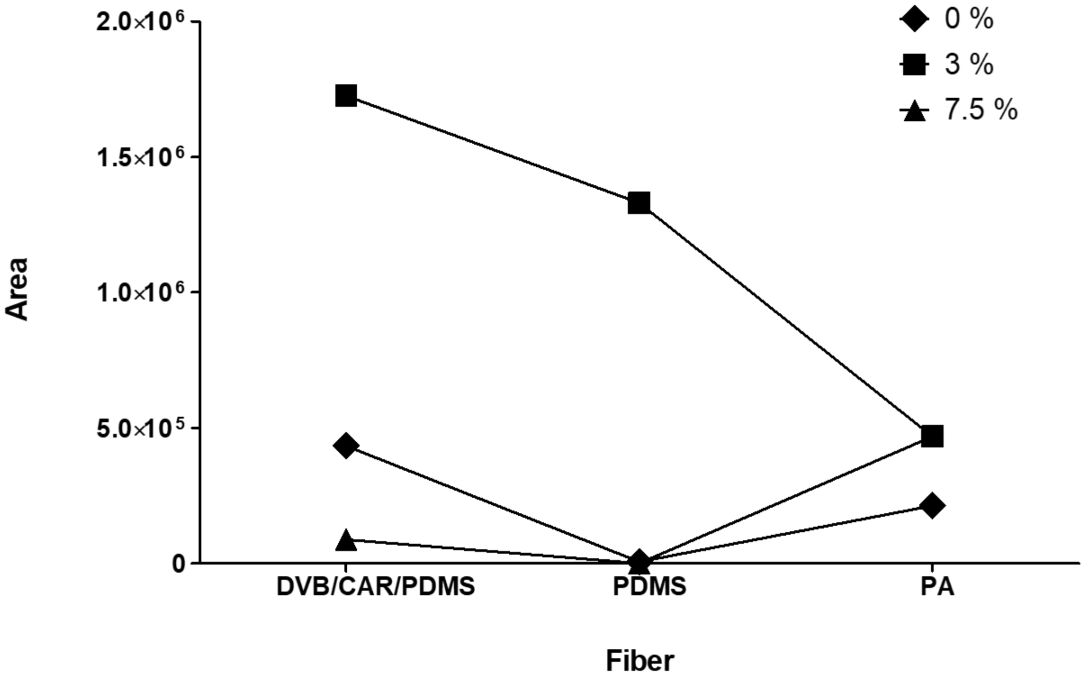

FIGURE 2-1 EFFECT OF SALT ADDITION FOR THE EXTRACTION OF 4-NP WITH DIFFERENT SPME FIBERS IN FORTIFIED SAMPLES WITH 1000 µG L^{-1}

The distribution constant between the sample matrix and the fiber coating increases when a soluble salt is added to the sample and so the sensitivity of the method. Aqueous solubilities of most organic compunds decrease when salt is added due to the -salting-out- effect that causes the analyte molecules to pass more easily from the sample to the HS and the fiber coating. Salt addition can increase or decrease the quantity of analyte extracted so it is necessary to evaluate the extraction efficiency by experimentation (Pawliszyn, 2010).

A temperature (60, 80, 100°C) and time (5, 10, 20, 30, 40 and 60 min) extraction study was performed using three types of fibers (PDMS, 100 µm; DVB/CAR/PDMS 50/30 µm and PA 85 µm). Each assay was carried out by triplicate and averages were used for the corresponding analysis. The results of the two-way ANOVA analysis (**Table 2.1**) show that there is no statistically significant difference between the parameters of time and temperature evaluated. The difference in the extraction lies in the polymeric fiber coating. **Figure 2.2** shows the profile of each evaluated fiber through a response surface graph as a function of the optimization variables (time and temperature). **Figure 2.2(a)** describes the response of the extraction of 4-NP with PDMS fiber, where the best extraction was accomplished in 40 min at a temperature of 80°C. The image shows that this type of fiber allows a better homogeneity in the extraction although thermal stability was not achieved. The response of the fiber DVB/CAR/PDMS is presented in **Figure 2.2(b)**. The best conditions for the extraction of 4-NP were using a extraction time of 20 min at 80°C. Unlike PDMS fiber requires a better thermal extraction control, a much higher response was obtained, and therefore it increases the quantity of 4-NP extracted. It has been reported that DVB/CAR/PDMS fiber offers the best efficiency of extraction and this fiber has shown a better performance with compounds with high molecular weights (Diaz et al, 2002). The PA fiber (**Figure 2.2(c)**) showed the lowest extraction capacity compared with the other two evaluated fibers. This is probably due to the interaction with the compound because of the polarity of the fiber. The conditions that allowed a higher sample extraction for this fiber were 30 min of extraction time at 60°C. The type of fiber is then, the most important factor showing an increase in the adsorption of the compound that is proportional to the response of the GC-MS.

TABLE 2-1. DETAILS OF THE TWO-FACTOR ANOVA TEST FOR ABUNDANCES OF 4-NP IN WATER SAMPLES IN RESPONSE TO THE FIBER AND TIME FACTORS. CONDITIONS: (1) TIME (5, 10, 20, 30, 40, 60 MINUTES) AND (2) FIBER (PDMS, 100 µM; DVB/CAR/PDMS 50/30 µM; PA 85 µM).

Source of variation	% of total variation	P value
Interaction	16.44	0.7672
Fiber	23.78	<0.0001
Time	5.77	0.0514

Source of variation	P value summary	Significant?
Interaction	ns	No
Fiber	**	Yes
Time	ns	No

Source of variation	Df	Sum-of-squares	Mean square	F
Interaction	40	8.208E+12	2.052E+11	0.8139
Fiber	8	1.187E+13	1.484E+12	5.887
Time	5	2.879+E12	5.758E+11	2.284
Residual	106	2.672+E13	2.521E+11	

ns: not significant

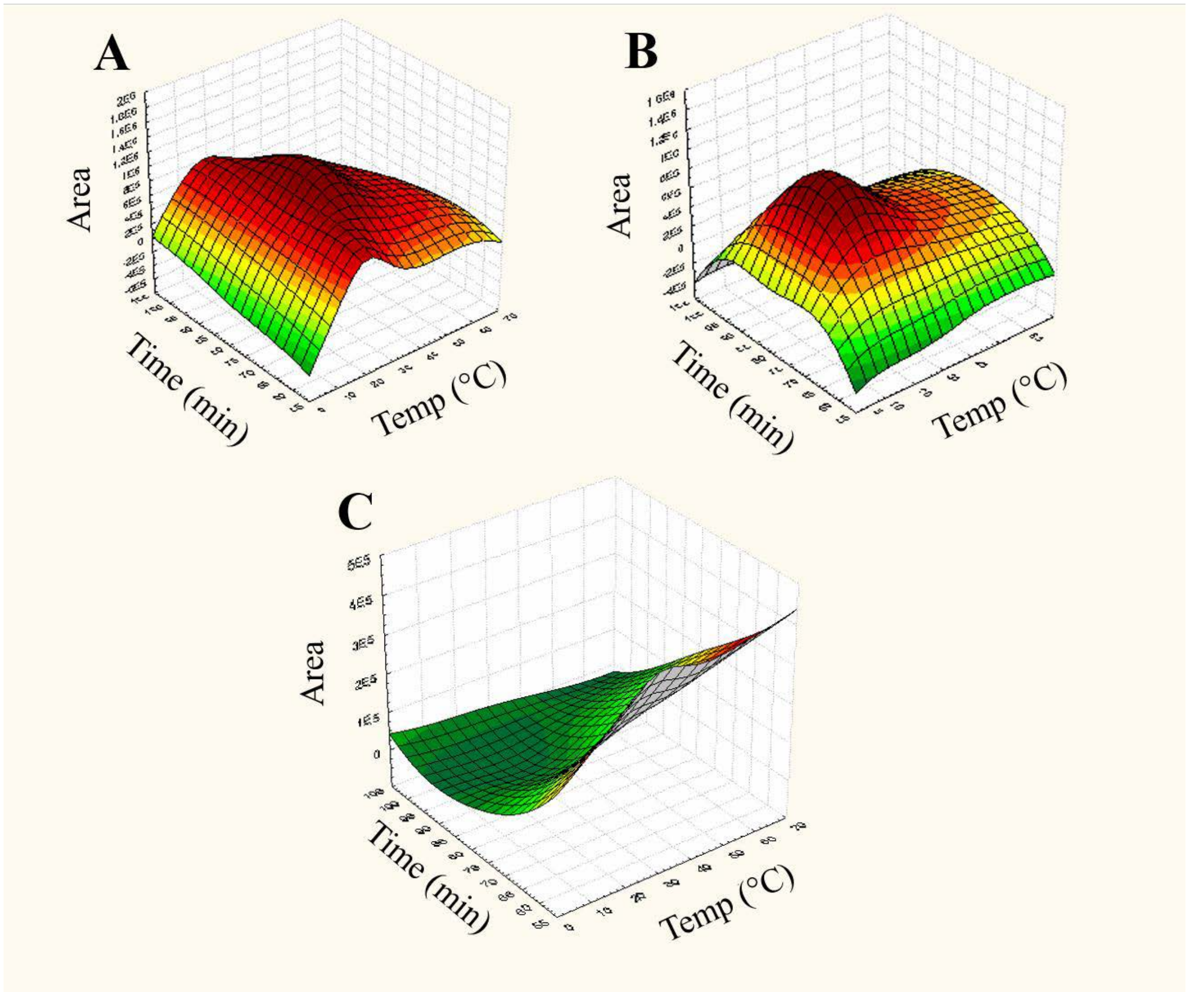

FIGURE 2-2. ESTIMATED OPTIMIZATION DESIGN BASED ON TEMPERATURE AND TIME WITH DIFFERENT SPME FIBERS. (A) PDMS, 100 µM, (B) DVB/CAR/PDMS 50/30 µM, (C) PA 85 µM

The selection of time extraction depends on the objective of the analysis because there is always an engagement between length, sensitivity and repeatibility. Equilibrium time is achieved when the maximum quantity of analyte has been extracted to the fiber (Pawliszyn, 2010). It is expected that compounds with high molecular weight, such as 4-NP, have larger equilibrium times due to the low diffusion between the gas phase and the fiber. The semivolatile property of the compound along with the derivatization process allow an adequate extraction. An equilibrium time of 20 min with the DVB/CAR/PDMS fiber was obtained. This type of fiber is characterized by its thick coating which has been reported to offer greater efficiency and sensitivity in the extraction process (Martinez et al, 2013).

Often a derivatization process is performed previous to the analysis to determine 4-NP. Derivatives tipically have higher distribution constants toward the coating and the volatility of the compound is increased. Therefore, the derivatization process is used to improve extraction and separation efficiency as well as increasing detection sensitivity (Pawliszyn, 2013). N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) is commonly used as derivatizing agent (Ros et al, 2015; Janicki et al, 2016; Rozalska et al, 2010). This study suggests the use of N-methyl-bis(trifluoroacetamide) (MBTFA) as a derivatizing agent because of the high reactivity of the obtained derivatives as well as the mild reaction conditions with products relatively inert, which prevents the gas chromatography column from damage, in addition it presents good reactivity with hydroxyl groups such as 4-NP (Zhou et al, 2007). It is possible that the choice of the derivatizing agent affects the sensitivity and capacity of extraction combined with the other considered parameters (e.g. temperature, coating of the fiber, exposure time, ionic strength). The silylation hyphenated in the derivatization process with BSTFA is difficult due to the hidrolisis of the reagent and silylmethylated derivatives in aqueous solutions (Vinas et al, 2010).

Optimal conditions were established as follows: one mililiter of water or sample, 3% of NaCl, 20 µl of 0.1 M HCl were added to a 10 mL sealed amber vial with gentle agitation. Subsequently the fiber DVB/CAR/PDMS was exposed to the headspace at 80°C for 20 min magnetically stirred at 600 rpm. A derivatization process was carried out after each sample extraction. After derivatization, the device was inserted into the GC injection port for 3 min at 230°C. The conditions of extraction were sufficient to ensure a detectable concentration of the analyte. The resolution of the chromatographic peaks was addecuate for the evaluation and quantification of the analyte of interest (**Figure 2.3**).

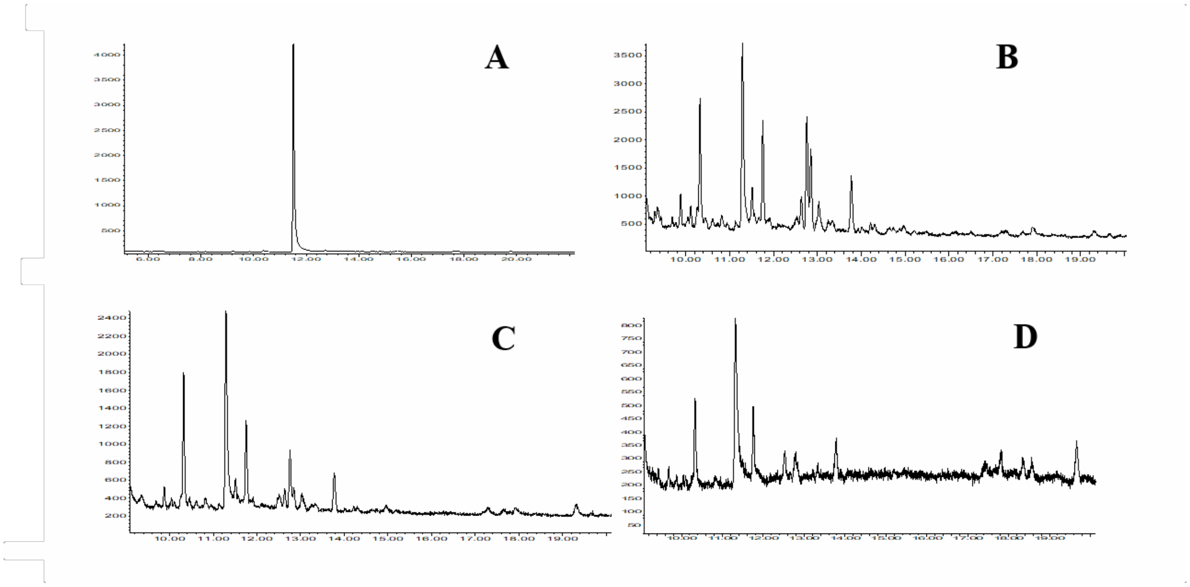

FIGURE 2-3. CHROMATOGRAMS HS-SPME-GC-MS (SIM MODE) OF WATER SAMPLES FROM DIFFERENT SOURCES IN MEXICO AND FROM A FORTIFIED SAMPLE. (A) FORTIFIED SAMPLE WITH 1000 µG L^{-1}, (B) MORALES LAKE, (C) SAN JOSE DAM, (D) COMMERCIAL BRAND 5.

2.3.2 VALIDATION OF THE METHOD

The method was validated in fortified samples to evaluate linearity, sensitivity, recovery percentage, repetibility and reproducibility. The calibration curves (**Table 2.2**) were linear over the working range (0.5 to 50 µg L^{-1}) and the correlation coefficient was above 0.99. LOD and LOQ were determined by a linear curve at low concentrations within a limit of confidence of 95% (Hubaux and Vos, 1970). The 4-NP concentrations were obtained based on blanks method (slope method). This method measures variations in the lowest values of the curve (where there is high uncertainty) to verify that the response of the obtained values are different than the blank. This is calculated from the intercept value (YB) plus the deviation that estimates random errors in the direction of the intersection (Sy/x),), YB + 3 Sy/x for LOD and YB + 10 Sy/x for LOQ. The 95% of the limit of confidence of the variation of the slope in the interpolation zone is added to the obtained value, but the final value contemplates a high uncertainty in the lowest values of the curve and assures the presence of the analyte and its quantification with an acceptable precision. Our study reported a LOD of 0.01 µg L^{-1}, this result is similar than the ones reported by Azzouz & Ballesteros (2014) and Sanchez-Avila (2017) with a LOD of 0.01 µg L^{-1} and 0.014 µg L^{-1} respectively. Another

SPME methods report higher LOD; for example Ros et al (2015) and Moeder et al (2000) with LOD of 0.076 µg L^{-1} and 0.2 µg L^{-1} respectively.

TABLE 2-2. VALIDATION PARAMETERS FOR HS-SPME-GC-MS ANALYTICAL METHOD IN WATER SAMPLES.

Calibration curve	RSD% Repeteability	RSD% Reproducibility	Repeteability*	Reproducibility*
50	7.2	6.23	18.83	12.55
25	5.3	4.47	20.90	13.93
2.5	25.4	23.84	29.57	19.71
1	14.1	20.03	33.93	22.63
0.5	15.4	20.97	37.66	25.11
R^2	**m**	**b**	**LOD***	**LOQ***
0.9923	835.09	531.84	0.01	0.15

Units: µg L^{-1} SD: standard deviation; CV: coefficient of variation; LOD: limit of detection; LOQ: limit of quatification; r: coefficient of correlation; m: sensibility expressed as a linear slope. *Acceptable RSD% (Horwitz 1982).

Table 2.3 shows a comparison between the developed method and other analytical techniques (Pei et al, 2017; Iparraguirre et al, 2011; Wu et al, 2016; Ballesteros et al, 2006; Pan and Tsai, 2008; Lopez-Darias, 2010; Bina et al, 2018). The present studys' method offers several advantages over other techniques. For example, only 1 mL of sample is needed and still low detection limits are obtained (0.01 µg L^{-1}). Additionally, the extraction time, considering the derivatization process, is limited to 30 min. This allows the analysis of multiple samples in short time. And, for the first time to our knowledge, the feasibility of MBTFA as derivatizing agent to determine 4-NP was demonstrated.

TABLE 2-3. CHROMATOGRAPHIC ANALYTICAL METHODS FOR DETERMINATION OF NONYLPHENOL IN WATER SAMPLES.

Compound	Sample volume (ml)	Extraction method	Derivatization	Analysis	Extraction time (min)	LOD	Reference
4-NP	1	SPME	MBTFA (100 µl)	GC-MS	30	0.01	This study
NP	250	SPE	-	HPLC-MS/MS	-	0.5	Martin et al 2014
NP	100	Continuous SPE	BSTFA (70 µl)+1%TMCS	GC-MS		0.01	Azzouz and Ballesteros 2014
NP mixture	10	SPME	-	GC-MS	30	0.13	Gong et al 2015
NP mixture	150	SBSE-PES	BSTFA(50 µl)+1%TMCS+Pyridine(125 µl)	GC-MS	720	0.076	Ros et al 2014
			-	LC-MS/MS	720	0.042	
4-NP	20	SPME	-	HPLC-DAD	60	0.6	Pacheco-FernandezHP et al 2016
4-NP	60	SPE	-	HPLC-DAD	40	0.34	Zhou et al 2017
4-NP	2500	MMF-SPME	-	HPLC-DAD	50	0.065	Pei et al 2017
NP mixture	15	SPME	-	GC-MS/MS	40	0.014	Sanchez-Avila 2017
4-NP	100	SBSE	BSTFA(2 µl)+1%TMCS	GC-MS	900	0.021	Iparraguirre et al 2011
4-NP	6	DLLME	BCEC-Cl (50 µl)	HPLC-MS	-	0.04	Wu et al 2016
4-NP	500	SPE	BSTFA(30 µl)+TMCS(30 µl)	GC-MS/MS	-	0.020 (LOQ)	Ballesteros et al 2006
4-NP	2	SPME	MTBSTFA (100 µl)	GC-MS	40	0.00385	Yi-Ping et al 2008
4-NP	20	PIL-SPME	-	GC-FID	60	0.0011	Lopez-Darias 2010
4-NP	5	DLLME	BSTFA+TMCS (10 µl + 10 µl)	GC-MS	-	0.00076	Bina et al 2018
NP	4	SPME	BSTFA (100 µl)	GC-MS	90	0.2	Moeder 2000

*Units: µg L^{-1} ; SPME: solid phase microextraction; DLLME: dispersive liquid liquid microextraction; SPE: solid phase extraction; SBSE-PES: stir bar sorptive extraction-polyether sulphone; MMF: multiple monolithic fiber; PIL: poly($VBHDI_m^+NTf^-_2$)MBTFA: N-methyl-bis-trifluoroacetamide; BSTFA: N,O-Bis(trimethylsilyl)trifluoroacetamide; TMCS: Trimethylchlorosilane; BCEC-Cl: 2-(11H-benzo[a]carbazol-11-yl) ethyl carbonochloridate; MTBSTFA: N-Methyl-N-tert-butyldimethylsilyltrifluoroacetamide; GC-MS: gas chromatography mass spectrometry; HPLC-MS: high performance liquid chromatography mass spectrometry; DAD: diode array detector; FID: flame ionization detector.

The feasibility of the SPME method was assessed by analyzing fortified samples with different concentrations. 4-NP obtained recovery values were of 94%, 106% and 118% for samples of 1, 50 and 100 µg L^{-1} respectively. This values are in the acceptable range of the validation guide used for the validation of the method (70-120% for concentrations between 10 µg L^{-1} y 100 µg L^{-1}) (AOAC/FAO/IAEA/IUPAC). The obtained values are similar to the ones reported by Bina et al (2018). Precision was measured as repeteability and reproducibility. Each point in the calibration curve was evaluated as the relative standard deviation (RSD%) of the area ratios. The compound (4-NP) has an acceptable RSD% which means that the developed method is reliable in different working days (Horwitz, 1982).

2.3.3 MONITORING OF 4-NP IN DIFFERENT SOURCES OF WATER IN MEXICO

It is difficult to determine the source of contamination in the 3 assessed scenarios: i) recreational water, ii) wastewater and iii) drinking water. However, the most common use of nonylphenol ethoxylate is in detergents (Kim et al, 2019; Priac et al, 2017); for example, in a study carried out in Taiwan, 41% of ninety domestic detergents contained from 0.2 to 21% of 4-NP (Chen et al, 2013). This suggests that the presence of 4-NP comes mainly from the use of detergents.

In **Table 2.4** the results of concentrations in the three analyzed scenarios are presented. Eighty-three percent of the analyzed samples presented concentrations above the LOD, of which sixty-five percent had concentrations above the allowed by the European Union (EU) (2 µg L^{-1}) and seventeen percent above concentrations recommended by the EPA (6.6 µg L^{-1}). Despite the fact that there is no statistically difference between the maximum concentrations in recreational and wastewater samples both were 2 times higher than the recommended by EPA and 6 times higher than the allowed by the EU. The recreational water presented concentrations ranging from 0.83 to 12.61 µg L^{-1} with a median of 3.61 µg L^{-1}; the wastewater from <LOD to 12.2 µg L^{-1} with a median of 3.88 µg L^{-1} and for drinking water from <LOD to 6.08 µg L^{-1} with a median of 2.48 µg L^{-1}.

TABLE 2-4. LOCATION OF THE WATER SAMPLES COLLECTED FROM DIFFERENT EXPOSURE SCENARIOS IN MEXICO.

Scenario	n	Median	Minimum	Maximum	P25	P75	% above reference EPA[1]	% above reference EU[2]
Recreational *	10	3.61	0.83	12.61	3.04	8.76	30	80
Wastewater *	14	3.88	<LOD	12.20	4.82	ND	7	57
Drinking water*	5	2.48	<LOD	6.08	ND	2.64	0	60

*Units: µg L^{-1}; LOD: 0.01 µg L^{-1}. [1] The Environmental Protection Agency (EPA) recommends concentration of 4-nonylphenol in fresh water less than 6.6 µg L^{-1}. [2] The Water Framework Directive of the European Union allows a maximum concentration in water of 2 µg $L^{-1}$of nonylphenol. Kruskal Wallis test-pos hoc Dunn, there is no statistically significant difference (p= 0.285).

Concentrations of 4-NP in recreational water could be attributed to tourism, people washing clothes and illegal water discharges in rivers (Fenet, 2003). The reported values have reasonable similarities with other countries (Pernica et al, 2015; Bina et al, 2018; Shao et al, 2005; Li et al, 2004; Sibabli et al, 2010; Martin et al, 2014; Wu et al, 2013; Sole et al, 2000; Loos et al, 2007; Fan et al, 2013; Maggioni et al, 2013). Nevertheless, these concentrations are much lower than the ones reported in Argentina (**Table 2.5**) (Babay et al, 2008). The presence of 4-NP in wastewater discharges could be associated with the use of detergents. Currently in Mexico the regulation of NP and its ethoxylates is null. So it is common to find these compounds in all types of detergents (industrial and domestic) because of its low cost and surfactant properties (Merreting-Bruns and Jelen, 2009; Perron and Juneau, 2011). It is important to acknowledge that concentrations in recreational water were higher than in wastewater discharges. In this context, the exposure of 4-NP by skin contact has to be considered, as previous studies have reported skin absorption of NP in humans and other species (Arukwe and Roe, 2008; Brunelli, 2018; Monteiro-Riviere et al, 2000; Sadakane et al, 2014). Levels of 4-NP in drinking water in this study were higher than the reported by other authors. Tap water in Mexico is not potable, instead water jugs are used. The jugs are washed with any kind of detergent each time before being refilled. A poor rinse may be the cause of the high concentrations of 4-NP in the reported samples. It is necessary to analyze drinking water before bottling in order to discuss this hypothesis. This ensures the need to monitor 4-NP and enforce the implementation of regulations in Latin America and Mexico.

TABLE 2-5. EXPOSURE ASSESSMENT OF 4-NP IN WATER SAMPLES FROM DIFFERENT SCENARIOS IN MEXICO COMPARED WITH DATA FROM DIFFERENT COUNTRIES.

Scenario	Country	n	Minimum	Median	Maximum	Reference
Recreational	Mexico	8	0.83	3.05	12.61	This study
	France	12	0.006	0.058	0.55	[52]
	China	15	0.1	1.3	7.3	[53]
	Korea	18	0.01	0.9	41.3	[54]
	South Africa	7	0.38	1.655	2.61	[55]
	Argentina	14	1730.0	2020.0	2390.0	[62]
	Spain	6	0.5	2	36.0	[56]
	China	21	0.093	0.161	0.332	[57]
Wastewater	Mexico	12	<LOD	3.79	12.20	This study
	Spain	4	6.0	142	289.0	[58]
	Argentina	6	2390	2550	2680	[62]
	Iran	9	0.42	0.807	2.12	[48]
	Belgium	10	0.25	0.745	2.5	[59]
	Italy	8	0.37	0.515	0.7	[59]
Drinking water	Mexico	5	<LOD	2.48	6.08	This study
	China	15	0.01	0.05	2.7	[53]
	Czech Republic	6	0.029	0.0335	0.045	[21]
	China	62	ND	0.027	0.558	[60]
	Italy	35	<0.0077	0.0149	0.084	[61]

*Units: µg L^{-1}

The following limitations have been considered when interpreting the results of this study i) the limited number of water samples collected in each scenario and ii) the samples were punctual or taken at a specific time and place, so they are not representative of each scenario. However, the objective of assessing 4-NP in different sources of water was accomplished and results present a general overview of the current situation and allow to assess the exposure levels in this region. The real source of contamination could not be defined; it is only possible to identify the final site in which the ecologic risk is presented. Mexico and Latin America do not have any regulations that establish minimum levels of NP in water. Therefore, there is scarce information about concentrations of this pollutant in surface and treated waters. The results presented in this investigation contribute with new information that could help to identify the sources of contamination, as well to evaluate the ecologic and human risk that the exposure to 4-NP represents. This information could not be ignored by politicians, academics and companies as the ecologic and human implications are of great importance. Continuous monitoring and new regulations in Mexico and America Latina according to the use of alquilphenols and its ethoxylates are imperative. Due to the lack of regulations in Mexico it is not possible to understand the implications of these values as there are no minimum permissible concentrations established. Likewise, the human risk for exposition cannot be classified and/or explained. This technique can support the continuous monitoring of this toxic in water samples.

2.4 CONCLUSIONS

A chromatographic analytical method was developed using HS-SPME combined with GC-MS for the extraction and quantification of 4-NP in water. Different parameters including type of fiber, extraction time, temperature and ionic strength were evaluated to optimize the analytical method. The feasibility of MBTFA as a derivatizing agent offering high sensitivity ensuring the detection of low concentrations was demonstrated. Having proved the sensitivity and efectiveness of the method, an analysis (HS-SPME-GC-MS) in water samples from different sources of water in Mexico was performed. In this study concentrations of 4-NP in water samples of recreational use and human consumption as well as in wastewater discharges were found. This pilot study detected 4-NP in eighty-four percent of the samples. Concentrations in recreational water, wastewater discharges and drinking water were up to 12.61 µg L^{-1}, 12.20 µg L^{-1} and 6.08 µg L^{-1} respectively, which suggests the need to explore potencial contamination sources. Environmental regulation measures are required to prevent the contamination in ecological receptors that could represent environmental harm. The ecological and health risk of 4-NP has been mainly related with endocrine disruption. As important concentrations of 4-NP in recreational water have been found in this study we recommend to consider skin exposure in humans and suggest further studies in this regard.

Concentrations of 4-NP found could cause a potential environmental impact and may represent a public health risk in Mexico. Thus, continous monitoring and regulations are desired and recommended. The results obtained in this study provide an efficient analytical tool for future studies of 4-NP in environmental samples.

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3. DETECTION OF 4-N-NONYLPHENOL IN DRINKING WATER FROM COUNTRIES WITH AND WITHOUT ENVIRONMENTAL REGULATION OF THE POLLUTANT.

SUBMITTED TO BULLETIN OF ENVIRONMENTAL CONTAMINATION TOXICOLOGY (BECT)

ABSTRACT

Nonylphenol ethoxylate is a compound mainly used for the fabrication of detergents, in environmental conditions it is transformed into a more toxic metabolite, Nonylphenol (NP). As limited information about risk assessment in humans for NP exposure is available, a risk assessment was performed. Concentrations of 4-n-NP in drinking water from Central Europe, United Kingdom and Mexico were determined to establish exposure concentrations and to perform a risk assessment by water intake in different regions and provide information that may be used as a basis for further studies. Samples were collected from water fountains in highly touristic places and from water jugs in countries where tap water is not drinkable. The analyte was quantified with gas chromatography-mass spectrometry after using solid-phase microextraction. Concentrations above the limit of detection were detected in 45% of the collected samples and 100% of these samples presented concentrations above the Directive on the Quality of Water intended for human consumption (0.3 µg L^{-1}). Our data presents a general view of the exposure levels and therefore, the environmental and health risks that these represent. According to the results, continuous monitoring and regulations of this pollutant are highly recommended to prevent exposure and thus, ecological and adverse health effects.

Keywords: Drinking water, Nonylphenol, Risk Assessment.

3.1 INTRODUCTION

Water quality research has focused on nutrients, microbial contaminants, heavy metals, and priority pollutants. Recently, a new type of pollutants ("emerging pollutants") has been recognized. Currently, more than 1036 emerging pollutants are listed in the European Aquatic Environment NORMAN Network (www.norman-network.net) affecting significantly the quality of water and causing potential public health and security problems (Bilal et al., 2019) like endocrine disruption. However, due to its recent detection and low concentrations (μg L^{-1}, ng L^{-1}), there is a gap in knowledge about its occurrence, fate, behavior, risk assessment, and ecological and human effects (Vargas-Berrones et al., 2020a). In addition, population growth has resulted in significant environmental impacts, with water being the most affected in terms of availability and quality (Peña-Guzmán et al., 2019). One of the most reported organic pollutant in wastewater, effluents, rivers, drinking water, sediments, and soil are the Alkylphenol Ethoxylates (APEs) (Belmont et al., 2006; Chen et al., 2013; Dong et al., 2015; Jie et al., 2017; Van Zijl et al., 2017). APEs are non-ionic surfactants widely used in the manufacturing of detergents, plastic additives, emulsifiers, pesticides, among others. Above 90-95% of APEs are removed from wastewaters with conventional treatment plants processes, however, the main problem is the formation of resistant metabolites that are classified as endocrine disruptors (Petrović et al., 2003). The APE most used is the nonylphenol ethoxylated (NPE), their use represents 80% of the production (Araujo et al., 2018) and their main property is the capacity to form micelles in solution which are formed because of the amphiphilic structure of the surfactant molecule which has a polar (hydrophilic) and a non-polar (hydrophobic) end. NPE main uses include the elaboration of detergents, emulsifiers, herbicides, pesticides, and in the production of paper, plastics, textiles, and paints (Ferrara et al., 2011). After the disposal of these products, NPE is degraded by microorganisms or ultraviolet light in environmental conditions transforming them into nonylphenol (NP) including mainly the 4-n-nonylphenol (4-n-NP) (Cheng et al., 2017). NP is classified as an endocrine disruptor which are external agents that interfere with the formation, elimination, transport, attachment, activity, or displacement of natural hormones that maintain homeostasis development, reproduction, and behavior (EPA, 1997). Endocrine disruptors are barely reviewed and regulated so there is scarce information regarding its occurrence, fate, and health impacts (Gavrilescu et al., 2015). Therefore, NP has been included as a priority pollutant by some agencies such as the Water Framework Directive of the European Union which allows maximum concentrations in surface water of 2 μg L^{-1} (European Union, 2013) and by the Environmental Protection Agency (EPA) stablishing a maximum concentration of 6.6 μg L^{-1} in surface water (EPA, 2005). The main exposure pathway for humans is through food and water intake which leads to bioaccumulation and biomagnification. Effects of NP exposure in animals have been previously reported in concentrations from 1 µg L^{-1} up to >195 µg L^{-1} (Lussier et al., 2000; Scaia et al., 2019; Tabassum et al., 2017), however effects in humans are still debated and require more investigation. Some studies suggest that the effects of NP exposure include decreased sperm count, an increase in the prostate, mama, ovarian and testicular cancer, reproductive malformations, immune deficiency, neurological effects, poor intellect development in children and psychological effects being the more vulnerable populations fetuses and newborns (Bolong et al., 2009; Tijani et al., 2016). The predicted no-effect concentration (PNEC) of NP has not been established because there is not enough toxicity data and the specific mechanism in organisms is still unclear (Bakke, 2003). Hence, the World Health Organization (WHO) suggests a maximum concentration of 0.3 µg L^{-1} in drinking water based in the precautionary values that comply with existing environmental quality standards (WHO, 2017). Regulations in developing countries that restrict levels of NP in water are null and the effects of the purification process are not well understood (Shannon et al., 2008). For this reason, it is important to establish monitoring strategies in water in order to contribute to the generation of regulations in this regard due to the toxicity associated with NP and the extensive use of NPEs. There is a major concern about the future and transport of NP through the environment and humans because of the continuous detection and identification of NP in water sources, thus the main objective of this study was to monitor the concentrations of 4-n-NP in drinking water from countries in Central Europe, United Kingdom (UK) and Mexico to assess human health risks.

3.2 MATERIAL AND METHODS

3.2.1 DRINKING WATER SAMPLING

Directed monitoring of water samples was performed in potential exposure sites in different countries of Central Europe, UK and Mexico (**Table 3.1**). Samples in Central Europe and UK were collected from drinking fountains located downtown or at highly touristic places; while water samples in Mexico were collected from different purified bottled water of commercial brands since tap water is note drinkable in this country. Samples were collected in one-liter plastic bottles previously rinsed with Milli-Q water, none detergent was used to prevent contamination. Immediately after sampling, they were stored in the dark at -20°C until its further analysis. The sample sites were positioned using a Garmin GPS in UTM coordinates.

TABLE 3-1. SAMPLES OF DRINKING WATER FROM DIFFERENT COUNTRIES IN CENTRAL EUROPE, UK AND MEXICO.

Sample	Coordinate	Place
1	BW Brand 1	San Luis Potosi, Mexico
2	BW Brand 2	San Luis Potosi, Mexico
3	BW Brand 3	San Luis Potosi, Mexico
4	BW Brand 4	San Luis Potosi, Mexico
5	BW Brand 5	San Luis Potosi, Mexico
6	45°26'02.1"N 12°20'14.1"E	Venice, Italy
7	45°28'47.6"N 12°13'18.0"E	Venice Mestre, Italy
8	41°53'28.9"N 12°29'34.9"E	Rome, Italy
9	43°46'24.3"N 11°15'19.4"E	Florence, Italy
10	48°08'14.3"N 11°34'29.6"E	Munich, Germany
11	52°30'58.6"N 13°22'46.5"E	Berlin, Germany
12	48°52'24.2"N 2°17'44.4"E	Paris, France
13	48°52'04.9"N 2°46'47.2"E	Marne-la-Vallée, France
14	41°23'52.0"N 2°12'40.8"E	Barcelona, Spain
15	40°24'56.2"N 3°41'03.3"W	Madrid, Spain
16	39°51'27.8"N 4°01'24.5"W	Toledo, Spain
17	53°24'31.7"N 2°58'56.8"W	Liverpool, United Kingdom
18	51°30'36.7"N 0°11'23.0"W	London, United Kingdom
19	53°28'39.6"N 2°13'54.9"W	Manchester, United Kingdom
20	55°56'59.7"N 3°12'09.0"W	Edinburgh, United Kingdom
21	55°51'37.3"N 4°15'19.6"W	Glasgow, United Kingdom
22	52°21'27.2"N 4°52'53.2"E	Amsterdam, Netherlands

3.2.2 4-N-NP QUANTIFICATION IN DRINKING WATER

4-n-NP quantification was performed based on the methodology described by Vargas-Berrones et al (Vargas-Berrones et al., 2020b). Solid-phase microextraction (SPME) with DVB/CAR/PDMS (50/30 µm stableflex divinylbenzene/carboxen/polydimethylsiloxane) sorbent was used for the extraction of 4-n-NP for both water samples and calibration curves. Stock standard solution of 4-n-NP (1000 µg mL^{-1}) was prepared in acetone and stored in the dark at -40°C until its further analyses. The standard work solution was prepared right before its use. The optimal conditions for extraction are established as follows: one mililiter of water and/or samples, 3% of NaCl, 20 µl of 0.1 M were added to a 10 mL sealed amber vial with gentle agitation. The fiber then was exposed to the headspace at 80°C for 20 minutes magnetically stirred at 600 rpm. A derivatization process was carried out to improve sensibility. This process consisted of exposing the fiber to the headspace above a solution of acetone (1 mL) with MBTFA (derivatizing agent) (100 µl) at 60°C for 10 min magnetically stirred at 600 rpm. After derivatization, the fiber was desorbed in the injection port of the gas chromatograph for 3 min at 230°C.

Samples and calibration curves were analyzed by gas chromatography (GC) (Agilent 6890) coupled to a mass spectrometry detector (MS) (Agilent 5975) in electron impact ionization mode (EI). The injection port was operated in splitless mode with a 0.75 mm liner without glass wool. Injection port temperature was fixed at 230 °C; helium was used as carrier gas at a pressure of 36 psi with a constant flow of 1 mL min^{-1}. The chromatographic separation was achieved through a HP 5ms (60 m x 0.25 mm x 0.25 µm) column (Agilent). The setting of the oven was as follows: first, the temperature was held at 90°C for 2 min, then it was increased to 180°C at a rate of 30°C min^{-1}, afterward up to 280°C at a rate of 1°C min^{-1}, and finally increased to 230°C at a rate of 30°C min^{-1} and held for 5 min. MS conditions were established for the source's temperature and its quadrupole at 230 and 150°C respectively. The tune parameters were: emission: 34.6; energy: 69.9: repeller: 26.6 and EMVolts: 1341. SCAN mode (50-500 m/z) was used to identify the compound, identification and quantification ions were selected for SIM mode. The identified fragment ions were 203/316 m/z. All determinations were performed in triplicate, thus the average values are reported. The lineal range ($r^2 = 0.99$) of the method was from 0.5 to 50 µg L^{-1}, the detection (LOD) and quantification (LOQ) limits were determined by blank signal and the obtained values were of 0.01 µg L^{-1} and 0.15 µg L^{-1}, respectively. The feasibility of the SPME method was assessed by analyzing fortified samples with different concentrations obtaining recovery values of 94% and 106% for samples of 1 and 50 µg L^{-1}, respectively. These values are in the acceptable range of the validation guide used for the validation of the method (70-120% for concentrations between 10 µg L^{-1} and 100 µg L^{-1}) (AOAC/FAO/IAEA/IUPAC, 2000). The precision was measured as repeatability and reproducibility with a coefficient of variation of 6.2 to 20.9% that, according to the concentrations, they remain in acceptable limits (Horwitz, 1982).

3.2.3 ESTIMATION OF RISK TO HUMAN HEALTH.

Human health risk assessment is based on reliable exposure pathways of contaminants (USEPA, 1989; USEPA, 1997). The expose doses through the ingestion pathway were calculated by use of **Eq. 1**, which were adapted from the US Environmental Protection Agency (USEPA, 1989):

$$D_i = \frac{C_w \, x \, IngR \, x \, EF \, x \, ED}{BW \, x \, AT} \qquad \text{Eq. 1}$$

Where:

Di = exposure dose through ingestion of water (mg/kg-day)

C_w= Concentration of contaminant in medium (mg L^{-1})

IngR = Ingestion rate (L day^{-1})

EF = Exposure frequency (days $year^{-1}$)

ED = Exposure duration (years)

BW = Body weight (kg)

AT = Average time (days)

The following data were considered: (i) **C_{medium}** is the concentration in water; either a measured or modeled value, (ii) **IngR** is the amount of water, that an individual ingests during a specific period; expressed in units of L day^{-1}, and (iii) **Table 3.1** of supplementary material establishes the different variables considered in the risk assessment for each studied group.

In June 2000 the EPA implemented several changes to carry out health risk assessments in infants (EPA, 2005). Due to the physiological and behavioral differences in each infant stage, we considered important to characterize the exposure based on each stage and to develop methods that allow the evaluation of different factors that influence the exposure. Therefore, age groups may represent a reliable guide to develop exposure scenarios. Health risk assessments consider different age groups focusing on infants as the most vulnerable group.

Consequently, the Exposure Factors Handbook establishes different values to assess exposure in the following groups: a) from 6 weeks to <1 year, b) from 1 < 6 years, c) from 6 < 21 years, d) adult 21 years < 31 years (EPA, 2011).

The hazard quotient (HQ) was calculated by **Eq. 2** to estimate the non-carcinogenic risk (USEPA, 1989):

$$H = {D_i}/{RfD} \qquad \text{Eq. 2}$$

Where :

Di (mg kg^{-1} day^{-1}): the exposure dose obtained from the Eq. 1.

RfD (mg/kg/day): the reference dose of the contaminant.

The ingestion reference dose (RfDi) values were obtained from the EPA with value of 0.1 mg/kg/day (Bakke, 2003).

3.3 RESULTS

Concentrations in drinking water from different countries are shown in **Table 3.2**. Results show that the obtained values are above concentrations established by current directives. Values above the LOD were detected in 45% of the collected samples, of which 100% presented concentrations above the Directive on the quality of water intended for human consumption (0.3 µg L^{-1}) (WHO, 2017). Reported values are similar to previous studies **(Table 3.3)** except the ones from Venice and Edinburgh. A risk assessment was conducted to calculate daily NP intake in children and adults **(Table 3.4).** The values obtained are lower than one, indicating that there is no potential risk of adverse health effects according to the values established by the EPA (0.1 mg kg-1 day-1). Regarding the estimation of the non-carcinogenic risk obtained by the HQ for water intake, the values are less than one, which indicates that there is no potential risk of adverse health effects (Bakke, 2003).

TABLE 3-2. CONCENTRATIONS OF 4-NP IN DIFFERENT SAMPLES OF DRINKING WATER.

Sample	Concentration
1	1.33
2	<LOD
3	2.32
4	2.64
5	6.08
6	40.29
7	3.99
8	<LOD
9	<LOD
10	<LOD
11	<LOD
12	<LOD
13	<LOD
14	<LOD
15	0.85
16	<LOD
17	<LOD
18	<LOD
19	<LOD
20	15.29
21	0.47
22	0.89

*Units: µg L^{-1}; LOD: 0.01 µg L^{-1}. NP was detected in 45% of the samples of which 27% show concentrations above the allowed by the EU (2 µg L^{-1}), 13% above concentrations recommended by the EPA (6.6 µg L^{-1}), and 45% above the Directive on the quality of water intended for human consumption (0.3 µg L^{-1}).

TABLE 3-3. CONCENTRATIONS OF 4-NP IN DRINKING WATER FROM DIFFERENT COUNTRIES.

	Country	n	Min	Median	Max	Reference
Drinking water	Mexico	5	<LOD	2.48	6.08	(Vargas-Berrones et al., 2020)
	China	15	0.01	0.05	2.7	(Shao et al., 2005)
	Czech Republic	6	0.029	0.0335	0.045	(Pernica et al., 2015)
	China	62	ND	0.027	0.558	(Fan et al., 2013)
	Italy	35	<0.0077	0.0149	0.084	(Maggioni et al., 2013)
	China	21	0.108	0.170	0.298	(Li et al., 2010)
	China	6	0.196	0.502	1.073	(Li et al., 2010)
	France	8	<LOQ	0.0159	0.0594	(Dupuis et al., 2012)
	China	8	0.0082	0.577 (media)	0.918	(Jie et al., 2017)
	Taiwan	18	0.017	0.032 (media)	0.195	(Cheng et al., 2016)
	Japan	9	0.016	0.076	0.078	(Toyo et al., 2000)

*Units: µg L^{-1}; LOD: limit of detection; LOQ: limit of quantification; ND: not detected

TABLE 3-4. NON-CARCINOGENIC RISK OF 4-NP EXPOSURE INGESTION FROM DRINKING WATER.

Country	Sample	Children from 6 wees to < 1 years old	Children from 1 to < 6 years old	Children/Adult from 6 to < 21 years old	Adult from 21 to < 31 years old
Mexico	Brand 1	5.20E-05	8.86E-05	2.34E-05	3.80E-05
	Brand 2	3.91E-07	6.67E-07	1.76E-07	2.86E-07
	Brand 3	9.07E-05	1.55E-04	4.08E-05	6.62E-05
	Brand 4	1.03E-04	1.76E-04	4.64E-05	7.53E-05
	Brand 5	2.38E-04	4.05E-04	1.07E-04	1.74E-04
Italy	Venice	1.58E-03	2.69E-03	7.09E-04	1.15E-03
	Venice Mestre	1.56E-04	2.66E-04	7.02E-05	1.14E-04
	Rome	3.91E-07	6.67E-07	1.76E-07	2.86E-07
	Florence	3.91E-07	6.67E-07	1.76E-07	2.86E-07
Germany	Munich	3.91E-07	6.67E-07	1.76E-07	2.86E-07
	Berlin	3.91E-07	6.67E-07	1.76E-07	2.86E-07
France	Paris	3.91E-07	6.67E-07	1.76E-07	2.86E-07
	Marne-la-Vallée	3.91E-07	6.67E-07	1.76E-07	2.86E-07
Spain	Barcelona	3.91E-07	6.67E-07	1.76E-07	2.86E-07
	Madrid	3.33E-05	5.67E-05	1.50E-05	2.43E-05
	Toledo	3.91E-07	6.67E-07	1.76E-07	2.86E-07
United Kingdom	Liverpool	3.91E-07	6.67E-07	1.76E-07	2.86E-07
	London	3.91E-07	6.67E-07	1.76E-07	2.86E-07
	Manchester	3.91E-07	6.67E-07	1.76E-07	2.86E-07
Scotland	Edinburgh	5.98E-04	1.02E-03	2.69E-04	4.37E-04
	Glasgow	1.84E-05	3.13E-05	8.27E-06	1.34E-05
Netherlands	Amsterdam	3.48E-05	5.93E-05	1.57E-05	2.54E-05

3.4 DISCUSSION

Previous studies have demonstrated that the occurrence of NP has increased significantly because of its great industrial demand (Silva et al., 2018). It is difficult to determine the source of contamination in water samples; however, the most common use of NPE is in detergents (Kim et al., 2019; Priac et al., 2017), i. e., 41% of 90 domestic detergents in Taiwan contained from 0.2 to 21% of NP (Huang et al., 2014). This may suggest that the presence of NP is mainly due to the use of detergents, tourism, people washing clothes, illegal water discharges in rivers, and inadequacy and lack of maintenance in drainage networks (Fenet et al., 2003; Gambolati et al., 2006). In countries where NP and its ethoxylates are not regulated, i. e. Mexico, it is common to find these compounds in detergents due to their excellent surfactant properties (Merrettig-Bruns and Jelen, 2009) and low cost (Perron and Juneau, 2011). In this country, water jugs are washed each time before being refilled, so a poor rinse may be the cause of concentrations of NP in drinking water. Also, conventional water purification processes do not remove endocrine disruptors like NP (Van Zijl et al., 2017) and NPE in water may be degraded into shorter ethoxylated chains in the treatment process and pollute drinking water (Soares et al., 2008). Besides, the chlorination process in water supply systems form byproducts like monochloro-NP (CNP) and dichloro-NP (DCNP) (Fan et al., 2013) that have been demonstrated to present estrogenic activity (Takemura et al., 2005).

The risk assessment performed shows that, in general, there is no risk for water intake according to the values established by the EPA (0.1 mg kg^{-1} day^{-1})(Bakke, 2003). Risk assessment regarding NP exposure has been previously reported in sludge (González et al., 2010; Kollmann et al., 2003; Roberts et al., 2006), surface water, wastewaters (Chen et al., 2014; Gao et al., 2014; Jin et al., 2014) and aquatic organisms (Lee et al., 2015; Pachura-Bouchet et al., 2006; Servos et al., 2003). Nevertheless, limited information about risk assessment in humans for NP exposure through food and water is available because of its high costs and the lack of scientific data in this area (Tijani et al., 2016). The United Nations Environment Programme establishes that the highest estimated value for human exposure through the environment (not in the vicinity of a NP manufacture) is of 5.31 x 10^{-3} mg kg^{-}

^{1}day^{-1}. The maximum intake combined from the air, water, and food exposure is set in 6.4 mg/kg/day, however, there is considerable uncertainty in the daily intake estimated. Therefore, it is difficult to determine accurate predictions in this regard (Bontje, 2002). This data considers only adults, despite it has been previously reported that children are more vulnerable to hormone impacts caused by environmental xenobiotics than adults (Longnecker et al., 2003; McElroy, 2008; Norgil Damgaard et al., 2002) because of the physiological significant differences such as constant increase of weight, higher respiration and ventilation range, higher relative consumption of water and food and faster brain development (Mishra and Vankar, 2002; Selevan et al., 2000). There is a critical window in fetal, neonatal, and postnatal development in humans (Lemasters et al., 2000) where susceptibility to adverse effects of environmental exposure is especially high and may lead to a permanent damage. Previous studies have shown that NP is ubiquitous in baby food presenting a daily intake from 0.23 to 0.65 µg kg-1 bw d-1 (Raecker et al., 2011). Even though these results do not represent a significant risk according to the values established by the EPA, the effects of exposure of low dose of endocrine disruptors during the development of humans have been underestimated (Welshons et al., 2006). On the other hand, previous studies have established that potencies of endocrine disruptors are much lower than natural hormones. Therefore, low concentrations exposures in humans have virtually no chance to compete with natural hormones in the unions of free receptors. This implies that health risks of endocrine disruptors are insignificants (Autrup et al., 2020). Considering that infants and babies are more vulnerable to hormonal effects and that their relative NP consumption through food and water is probably higher than adults, a potential risk for this population is considerably higher because a reference dose specific for infants has not been established. Some limitations were considered when interpreting the results of this study. 1) Only 4-n-NP was considered because it is commonly used as a reference due to its commercial availability (Calafat et al., 2005) but it is important to acknowledge that NP is a mixture of approximately 20 para-substituted isomers with different branched alkyl chains (Ieda et al., 2005; Ruß et al., 2005; Thiele et al., 2004; Wheeler et al., 1997) and with intermediate structures compounds between NPE and NP that make the mixture more toxic (Jin et al., 2014). It also has to be considered that NP isomers may elute together due to the characteristics of the method. Previous studies have reported that 4-n-NP only represents 2.2% of the total mass in water and represents only 26% of the total risk of NPE and their transformation products (Fenner et al., 2002). Moreover, it has been demonstrated that NP isomers have relevant differences between them regarding disruptive endocrine activity so it is not acceptable to take any isomer as a general reference to establish models, activity, and structure relations or risk assessments (Preuss et al., 2006). Therefore, to achieve a complete risk assessment, it would be important to identify all isomers in the sample. Even though our study just considers 4-n-NP it provides a reference point and allows to establish minimum exposure concentrations.

2) Other pollutants classified as endocrine disruptors besides NP may be found in drinking water, consequently, it may be practically impossible to predict health problems caused by each pollutant since they can act independently or in synergy with some other. For example, Bisphenol A (BPA) is a monomer used for polycarbonates and epoxy resins production. This compound is utilized as a coating for food cans, water containers, water pipes, reusable milk containers, food storage vessels, and baby bottles. It has been reported that BPA possesses estrogenic activity and is considered an important organic pollutant because of its incomplete polymerization during manufacture and because the increase of temperature in the process may cause the compound to leach in food and beverages (Markey et al., 2001). Phthalates (PEs) are also endocrine disruptors that are mainly used as plasticizers to impact flexibility, workability, and durability of polymers; and in the manufacture of paints, adhesives, dyes, and cosmetics. These compounds may be incorporated in food and water through packaging, bottles, and manufacturing processes because there is no chemical bonding with the polymers (Serodio and Nogueira, 2006).

3.5 CONCLUSIONS

This study aims to provide a reference to establish minimum exposure concentrations and to show a risk assessment from NP exposure through water intake. An exposure scenario of 4-n-NP in drinking water is presented in countries with current regulations (Central Europe and UK) and countries without any regulation in this regard (Mexico). Our results show that NP was detected in 45% of the samples of which 45% presented concentrations above the Directive on the quality of water intended for human consumption. Although these results do not represent a significant risk for the consumption of NP through water intake there is no consensus above the scientific community about this issue, furthermore, it is important to bear that current parameters are considered for adults, and intake in children may represent a greater risk. Efforts in investigation and strategies to promote consciousness of the impact of NP as a pollutant are required to obtain a responsible approach to the use and handling of NP and its ethoxylate. The search for sustainable quality in the environment to protect public health and the environment is a compulsory long-term challenge that is shared by most modern societies and civilizations around de world. Good practices and environmental policies are needed to mitigate potential health and ecologic risks before reusing water to make it drinkable. Therefore, the implementation of strategies to promote awareness of the negative impacts of endocrine disrupter is imperative. Additionally, environmental regulations and continuous monitoring are required to address the contamination in ecological receptors that could represent significant effects in human health. The results of this study provide new and useful information that could be used as a basis for further studies.

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4. COMPARISON OF THE SURFACTANTS NONYLPHENOL ETHOXYLATE AND ALKYL POLYGLUCOSIDE IN DETERGENTS.

SUBMITTED TO ENVIRONMENTAL TECHNOLOGY & INNOVATION

ABSTRACT:

Detergents are one of the most common pollutants in water causing environmental problems like foam generation and toxic effects in biota. Nonylphenol ethoxylate (NPE) is the main surfactant used in detergent formulations. In the environment, NPE biodegrades forming the metabolite nonylphenol classified as an endocrine disruptor. Regulations in developed countries prohibit or limit their use due to the adverse effects caused by the exposure to nonylphenol. However, in developing countries there are any regulations in this regard. The substitution of NPE as a surfactant in detergents is of a great importance; therefore, the objective of this investigation is to evaluate the substitution of NPE with alkyl polyglucoside and establish the feasibility of the substitution according to physical and chemical properties, cleaning performance, and antimicrobial properties. Detergent with alkyl polyglucoside showed lower surface tension, critical micelle concentration, and viscosity, benefiting soil removal, improving cleaning processes, minimizing economic and environmental costs, and providing a cleaner and more efficient production. Appropriate antimicrobial properties in the proposed detergent were found without toxicity problems, andregarding the detergency test, it is observed that each detergent works better for different stains. Therefore, results show that alkyl polyglucoside may be an adequate substitute, because it offers similar and/or better properties than detergents formulated with NPE, eliminating exposure risks.

Keywords: nonylphenol ethoxylate, alkyl polyglucoside, detergents

4.1 INTRODUCTION

Nowadays, the chemical industry represents one of the most important economic sectors worldwide, and even though there are important developments, the introduction of new technologies is still occurring (Ruß et al., 2005). Rapid industrialization and economic development have caused an exponential population and urbanization growth (Chen, 2018; Zafra et al., 2015) which leads to higher consumption of natural resources and environmental pollution (Li and Yu, 2011; Prospects), 2014; Zhang et al., 2015). Domestic and industrial wastes and sewage are disposed without treatment or improperly confined (Varjani et al., 2020), therefore, the development of sustainable practices is a current pressing challenge (Hoornweg, 2012). Industrial processes are known as the main source of environmental pollution because they contribute with hazardous materials to the health and the environment (Jovanic, 2010; Nie et al., 2017). Among industrial pollutants, those that are highly produced and used in large quantities are of interest, for example, compounds used in detergent formulation (Abd-Allah and Srorr, 1998). Sixty billion dollars have been used for detergent production (Giagnorio et al., 2017) and in 2014 the surfactant market in the world was of more than $ 33 millions of dollars (Karray et al., 2016). Detergents contain surfactants among other materials like builders, boosters, fillers and auxiliary compounds (Sirisattha et al., 2004). Effects caused by detergents in the environment have been previously reported, for example, the production of a layer of foam in aquatic environments that reduces the oxygen exchange rate from the air to the water resulting in a decrease of oxygen available and causing mass death of aquatic organisms (Sri et al., 2005). On the other hand, detergents usually use a mixture of alkylphenols (with different chain lengths and isomers) predominating nonyl and octyl groups (Scott and Jones, 2000). Nonylphenol ethoxylate (NPE) is a yellow viscous liquid that is produced by the refining process of petroleum (Vazquez-Duhalt et al., 2005) and its technical preparation is a complex mixture of isomers (Soares et al., 2008). The NPE is an efficient, economic, and versatile surfactant, used in detergents for more than 40 years (Yu et al., 2008) and represent 80% of its demand (Féliz, 2009; Vazquez-Duhalt et al., 2005). Even though NPE meets primary biodegradation, the product obtained (Nonylphenol-NP) is more persistent to further degradation (Karsa, 1999; Ying et al., 2002) and it is classified as an endocrine disruptor causing adverse effects in organisms like change of sex, hermaphroditism, reduction in testosterone elimination, fertility problems, mutations, deformities, increased mortality (Naderi et al., 2014), inhibition of gonadal and ovaries development, and low testicular mass (Huang et al., 2014). Due to high concentrations of NP found in different water bodies (Babay et al., 2008; Bina et al., 2018; DeArmond and DiGoregorio, 2013; Jardim et al., 2012; Shao et al., 2005; Vargas-Berrones et al., 2020b) regulations in developed countries had been established. The European Union (UE) with the Directive 2003/53/EC (European Union, 2003) establishes that this compound "…may not be placed on the market or used as a substance or constituent of preparations in concentrations equal or higher than 0,1 % by mass…", Directive 775/2004(02/2076) (Journal, 2004) prohibits the use of NPE in pesticides formulations, and Directive 2013/39/EU (European Union, 2013) enlists NP as a priority pollutant and allows maximum concentrations of 2 µg L^{-1} in water bodies. In the United States, the Environmental Protection Agency (EPA) recommends concentrations of NP in surface water lower than 6.6 µg L^{-1} and in saltwater lower than 1.7 µg L^{-1} (EPA, 2005). Canada enlists NP as a toxic substance in the Canadian Environmental Protection Act and the Rotterdam Convention classifies it as a severely restricted compound. However, there are still countries with a lack of regulation such as counries in Latin America (Vargas-Berrones et al., 2020a). Therefore, NPE substitution is an increasing and current trend. Usually, it is substituted with alcohol ethoxylates, less effective surfactants but safer for the environment due to its rapid biodegradation (Yu et al., 2008). On the other hand, natural surfactants from renewable sources have been introduced (Scott and Jones, 2000) such as fatty acid esters of sugars, fatty acid esters, amino acid amides, lecithin, and various types of sugars (Deleu and Paquot, 2004; Holmberg, 2001). Sugar-based surfactants have gained more attention because of their great advantages like their cleaning performance, consumers´ health, and environmental compatibility (Fina Uzwatania, 2017). Alkyl Polyglucosides (APGs) are sugar-based surfactants with synergic effects, low irritation potential, full biodegradation, and non-toxic (Wasilewski et al., 2016; Yu et al., 2008). More evidence of sustainable alternatives to change technology gradually is needed mostly in developing countries where higher concentrations and health effects by exposure are found (Vargas-Berrones et al., 2020a). Therefore, the objective of this study is to compare the physical and chemical properties of detergents with NPE and APG respectively and evaluate its possible substitution.

4.2 MATERIALS AND METHODS

4.2.1 PREPARATION OF DETERGENTS

Liquid detergents were prepared following the recommendations in ISO 6330:2012 (**Table 4.1**). Solutions were stirred for 30 minutes at room temperature for the corresponding physicochemical analysis (Stüpel, 1954). Raw materials characterization was performed with an electronic nose (Heracles 2 Alpha Mos).

TABLE 4-1. LIQUID DETERGENTS PREPARED ACCORDING THE RECOMMENDATIONS IN ISO 6330:2012.

Detergent Nonylphenol Ethoxilated (DNP)	**Concentration (%)**	**Detergent Alkyl Polygucoside (DAPG)**	**Concentration (%)**
Nonylphenol Ethoxilated	12.6*	Alkyl Polygucoside	10.5*
Fatty alcohol	10.0	Fatty alcohol	10.0
Builder	5.5	Builder	5.5
Enzymes	0.001	Enzymes	0.001
NaOH	0.5	NaOH	0.5
Water	71.399	Water	73.499

* The difference in percentage is due to the density provided by the supplier of the raw material.

4.2.2 INSTRUMENTAL MEASUREMENTS

Physicochemical properties like surface tension, critical micelle concentration (CMC), and viscosity were determined. Surface tension was performed following the Spanish Standard UNE 55-501-90 (Española, 1990) with a solution of 1 g L^{-1}, test temperature of 20ºC using a tensiometer (MGW-LAUDA Hucoa-Erlôss) with a 4 cm diameter platinum foil. CMC was calculated by measuring the change in surface tension with the variation of concentration in the solution. Viscosity was determined following the ASTM D-445-06 (D445-06, 2006) using a calibrated viscosimeter, a holder, a bath at a controlled temperature where the temperature may not vary in more than 0.02 ºC, a glass thermometer accurately calibrated (0.02º C), and a chronometer that allows lectures with 0.1 seconds of discrimination. Also, detergency tests were performed, such as foaming power, wetting power, and decontamination rate. The foaming power was determined following the Spanish Standard UNE-EN 14371 (14371, 2005) using a graduated test tube and a gear pump with outlet flow of 0 L h^{-1} up to 280 L h^{-1} and that allows flow adjustment of 200 L h^{-1}. Results were reported in height of produced foam of a 1 g L^{-1} solution at 50 ± 2°C measuring at 30 seconds, 3 minutes, and 5 minutes. The wetting power by immersion was performed according to the ISO 8022:1990 using a cotton woven disc DIN 53901. The cotton discs were immersed in a solution of known concentration to establish the wetting time (time in which the cotton disc starts to drown). Detergency tests were determined by the ISO 6330:2012 using a front-loading horizontal rotating drum-type machine (Type A), polyester fabric squares of 20 ± 4 cm by 20 ± 4 cm with a mass per area of 310 ± 20 g cm^{-3} and individual weight of 50 ± 5 g. Test cloth materials with artificial soils were acquired from the Center for Test Materials B.V. (**Table 4.2)** and tested using a concentration of detergent of 8 g L^{-1}. Detergency power is reported as a decontamination rate and was measured with a reflectance photocolorimeter (GretagMacbeth-Color i5). Minimum inhibitory concentration was evaluated using four different strains to establish a wide range of test-organisms: (i) *Staphylococcus epidermis*, (ii) *Escherichia coli*, (iii) *Candida sp*., and (iv) *Pseudomonas aeruginosa* grown in agar coliform at 37ºC for 24 hours and were determined following the turbidimetry methodology (ISO 20776-1) using as liquid culture medium sterile phosphate-buffered saline (PBS) and measuring with a microplate reader Infinite M200, Tecan Software Magellan®.

TABLE 4-2. TEST CLOTH MATERIALS FOR CLEANING PERFORMANCE TESTING

Product code	Description
C-BC-01	Tea for medium/high temperatures
C-BC-02	Coffee
C-BC-03	Tea for low temperatures
C-S-01	Blood (aged)
C-S-02	Cocoa
C-S-03	Wine (aged)
C-S-08	Grass
C-S-12	Black currant juice
C-S-15	Bill(blue)berry Juice
C-S-28	Rice starch, colored
C-S-44	Chocolate drink, pure
C-S-103	Wine, not aged
C-05	Blood, milk, ink
C-12	Pigment, oil, low milk content
PCN-1	White (standard)

4.3 RESULTS AND DISCUSSION

4.3.1 PREPARATION OF DETERGENTS

The preparation of the detergents was following the recommendations established in ISO 6330:2012 (6330:2012, 2012) using nonylphenol ethoxylate and alkyl polyglucoside respectively. Fatty alcohol was added as a complementary surfactant; a builder was used for water softening and as a preservative to improve detergent performance (Lim et al., 2019), enzymes (biodegradable and non-toxic compounds) were included to reduce the use of large quantities of chemicals and to improve cleaning capacity (Hameed, 2013), sodium hydroxide was used as an alkali to maintain pH and favor the cleaning process (Chiplunkar et al., 2017). **Figures 4.1** and **4.2** show the characterization of NPE and APG respectively.

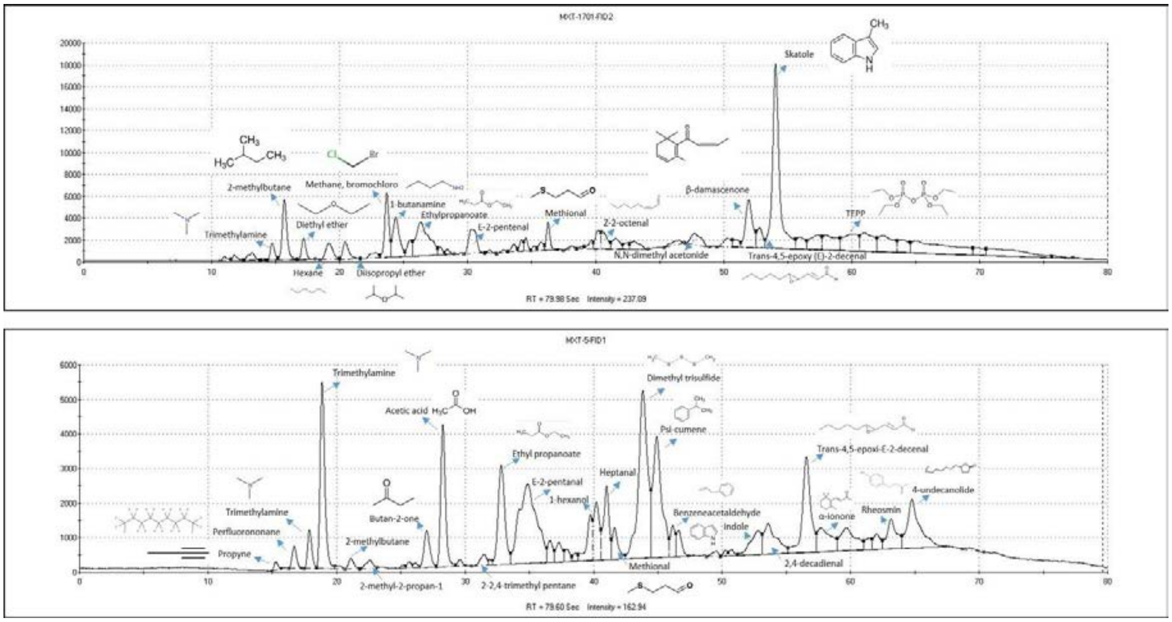

FIGURE 4-1. NPE CHARACTERIZATION.

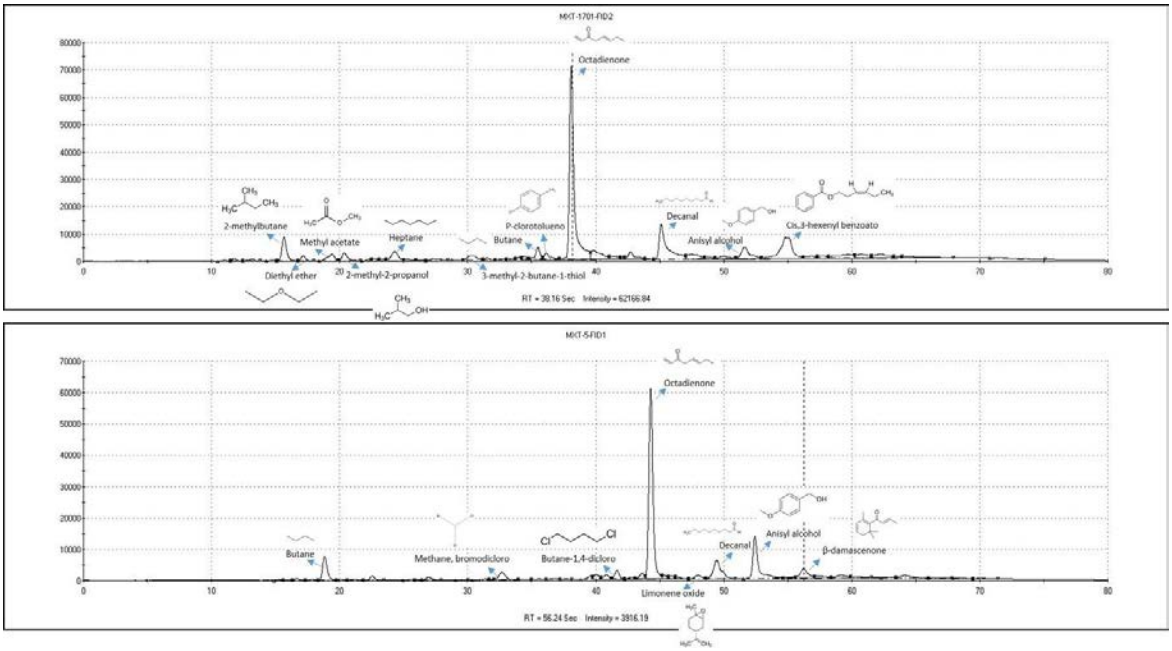

FIGURE 4-2 APG CHARACTERIZATION.

4.3.2 INSTRUMENTAL MEASUREMENTS

Table 4.3 shows the results of surface tension, wetting power, foam power, and viscosity. Regarding surface tension, the detergent with alkyl polyglucoside (DAPG) offers a lower surface tension benefiting soil removal (Mousavi and Khodadoost, 2019). CMC was determined by the change in surface tension, DAPG shows lower CMC than DNP, meaning that less detergent is needed to achieve the maximum formation of micelles, and therefore improving cleaning processes and minimizing economic and environmental costs (Singh et al., 2018). Regarding viscosity, detergents that present high viscosity may hinder the production process and kinetically limit dilution in water (Calero et al., 2017). Results show that DAPG has 22 times lower viscosity offering a cleaner and more efficient production. On the other hand, detergents were analyzed without antifoaming agents and DAPG presented a foam height 3 times higher than DNP. This behavior is expected because it has been previously demonstrated that compounds with lower surface tension favor foam generation because of the decrease in energy of the molecular interface (Chiplunkar et al., 2017). However, previous studies show that APG foam height decreases as the alkyl chain length increases and increases gradually in function of the concentration, so APG may be selected according to the foam stability desired (Chiplunkar et al., 2017; El-Sukkary et al., 2008). Currently, foam elimination in discharges is desired due to the growing concern for the environment. Therefore, natural antifoaming agents may be used in the production process to control foam generation (Schilling and Zessner, 2011). On the other hand, the wetting power of liquid detergents is related to the efficiency of soil removal (Moulay et al., 2005) and even though DNP presents better wetting power it may be observed that as the concentration of the DAPG increases the wetting time decreases Therefore, the wetting power is expected to be much higher since 8 g L^{-1} was used for the detergency test.

TABLE 4-3. PHYSICAL AND CHEMICAL PROPERTIES OF DETERGENTS WITH NPE AND APG.

<table>
<tr><th></th><th colspan="12">DNP</th><th colspan="12">DAPG</th></tr>
<tr><td rowspan="2">Surface tension (mN/m)</td><td colspan="3">100%</td><td colspan="3">50%</td><td colspan="3">20%</td><td colspan="3">5%</td><td colspan="3">100%</td><td colspan="3">50%</td><td colspan="3">20%</td><td colspan="3">5%</td></tr>
<tr><td colspan="3">37.9</td><td colspan="3">32.0</td><td colspan="3">30.9</td><td colspan="3">30.7</td><td colspan="3">30.2</td><td colspan="3">29.8</td><td colspan="3">25.5</td><td colspan="3">25.4</td></tr>
<tr><td rowspan="2">Wetting power (sec)</td><td colspan="3">5 g L^{-1}</td><td colspan="3">4 g L^{-1}</td><td colspan="3">3 g L^{-1}</td><td colspan="3">2 g L^{-1}</td><td colspan="3">5 g L^{-1}</td><td colspan="3">4 g L^{-1}</td><td colspan="3">3 g L^{-1}</td><td colspan="3">2 g L^{-1}</td></tr>
<tr><td colspan="3">37</td><td colspan="3">63</td><td colspan="3">97</td><td colspan="3">137</td><td colspan="3">163</td><td colspan="3">219</td><td colspan="3">268</td><td colspan="3">305</td></tr>
<tr><td rowspan="2">Foaming power (ml)</td><td colspan="4">30 sec</td><td colspan="4">3 min</td><td colspan="4">5 min</td><td colspan="4">30 sec</td><td colspan="4">3 min</td><td colspan="4">5 min</td></tr>
<tr><td colspan="4">205</td><td colspan="4">164</td><td colspan="4">153</td><td colspan="4">690</td><td colspan="4">620</td><td colspan="4">550</td></tr>
<tr><td>Viscosity (mpoise)</td><td colspan="12">445.74</td><td colspan="12">20.38</td></tr>
</table>

Results of the detergency test are shown in **Table 4.4**. It is observed that each detergent works better in different types of stains, i.e. DNP offers a higher decontamination rate for coffee and te stains but DPAG shows a better decontamination rate for juice stains. Other stains like grass, rice starch, pigment, oil, wine, cocoa, among others show similar decontamination rates. Therefore, the detergent capacity of APG as surfactant may be considered as satisfactory, it is only a matter of the evaluation and the analysis of proportions in formulations according to their use (laundry industries, metal mechanical industries, etc.). These results are similar to previous studies where the use of APG in detergent formulations show a better decontamination rate in different types of fabric, significantly reduces surface tension and CMC allowing better penetration of the detergent in textiles and achieve better soil removal (Karthick et al., 2018; Pukale et al., 2017). However, the evaluation of other alternatives of natural surfactants is needed. Methyl ester sulfonate (MES) has proven stability in hard water and meets cleaning standards (Tai et al., 2018). **Table 5** presents antimicrobian properties results. As it has been previously reported (Dembitsky, 2005; Matsumura et al., 1990; Rauter et al., 2005) DAPG presents appropriate antimicrobial properties without toxicity problems, unlike DNP.

TABLE 4-4. RESULTS OF DETERGENCY TEST FOR DETERGENTS WITH NPE AND APG.

			DNP	**DAPG**
No.	**Product code**	**Description**	**% of decontamination rate**	**% of decontamination rate**
1	C-BC-01	Tea for medium/high temperatures	4.09	0.24
2	C-BC-02	Coffee	3.23	0.81
3	C-BC-03	Tea for low temperatures	4.98	1.19
4	C-S-01	Blood (aged)	8.05	8.43
5	C-S-02	Cocoa	4.92	6.68
6	C-S-03	Wine (aged)	2.74	3.40
7	C-S-08	Grass	21.23	19.69
8	C-S-12	Black currant juice	16.33	23.88
9	C-S-15	Bill(blue)berry Juice	14.79	24.74
10	C-S-28	Rice starch, colored	17.28	17.78
11	C-S-44	Chocolate drink, pure	1.87	6.49
12	C-S-103	Wine, not aged	0.94	11.46
13	C-05	Blood, milk, ink	0.34	0.50
14	C-12	Pigment, oil, low milk content	9.98	6.32

TABLE 4-5. ANTIMICROBIAN PROPERTIES RESULTS OF DETERGENTS WITH NPE AND APG.

		DNP			DAPG		
	Time (seg)	**10%**	**30%**	**50%**	**10%**	**30%**	**50%**
S. epidermis	0	0.082	0.098	0.093	0.115	0.14	0.178
	7167	0.083	0.131	0.103	0.102	0.136	0.168
	14335	0.085	0.129	0.103	0.103	0.136	0.167
	21503	0.093	0.142	0.115	0.105	0.137	0.167
	28671	0.093	0.144	0.114	0.105	0.137	0.167
	36262	0.092	0.145	0.114	0.106	0.137	0.167
E. coli	0	0.088	0.083	0.108	0.106	0.135	0.192
	7167	0.097	0.091	0.109	0.093	0.13	0.182
	14335	0.104	0.09	0.108	0.094	0.131	0.181
	21503	0.11	0.091	0.107	0.095	0.132	0.182
	28671	0.119	0.09	0.107	0.096	0.134	0.183
	36262	0.115	0.091	0.107	0.098	0.135	0.183
Cándida	0	0.091	0.094	0.093	0.115	0.142	0.193
	7167	0.09	0.109	0.122	0.101	0.137	0.184
	14335	0.094	0.112	0.121	0.103	0.138	0.184
	21503	0.095	0.116	0.114	0.106	0.14	0.185
	28671	0.096	0.116	0.114	0.109	0.142	0.186
	36262	0.096	0.117	0.114	0.112	0.143	0.186

TABLE 4-6. ANTIMICROBIAN PROPERTIES RESULTS OF DETERGENTS WITH NPE AND APG.

Pseudomonas aeruginosa	0	0.119	0.13	0.091	0.113	0.14	0.188
	7167	0.124	0.149	0.095	0.1	0.135	0.173
	14335	0.123	0.151	0.096	0.104	0.139	0.174
	21503	0.126	0.15	0.095	0.105	0.141	0.174
	28671	0.128	0.149	0.093	0.106	0.142	0.175
	36262	0.127	0.15	0.094	0.107	0.142	0.176

Currently, to our knowledge there are not other studies regarding detergents formulations with APG. Also, the quantity of nonionic surfactants used in detergent industries is not clear, thus, a precise evaluation and environmental risks associated are difficult to establish. On the other hand, previous studies have found concentrations of NPE in detergents from 0.2 to 21%. Additionally, the NPE market is estimated to be worth USD 928.89 million by 2027 registering a growth rate of 7.3% for the period from 2020 to 2027 (Research, 2020). The growing demand for NPE for fertilizers and paint industry, in addition to the detergent industry, will generate new opportunities for the market and in the absence of regulations in some countries (i.e. Latin America) environmental effects and associated risks are expected. Even though it is well known all the risks and negative effects of the use of NPE in the industry and that APG as a natural surfactant presents some advantages, it is still a challenge to persuade industries to replace conventional surfactants because of the costs that these represent (up to 50 times higher than synthetic surfactants) and the environmental concern (Deleu and Paquot, 2004).

4.4 CONCLUSIONS

A detergent was developed using an APG in substitution of NPE. Similar or better properties were observed offering some advantages. For example, the decrease of surface tension and CMC showing a better penetration of the detergent in the fabrics tested. Also, APG demonstrated better or similar detergent capacity in the different types of stains evaluated and showed effective inhibition of bacterial growth of the four strains studied. Although an increase in NPE demand is expected, it is also expected that the trend towards substitution of NPE increases because of the multiple benefits that natural surfactants offer in terms of availability, performance and environmental health.

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DISCUSIÓN DE RESULTADOS

Los contaminantes emergentes (CEs) presentan un nuevo reto global acerca de la calidad del agua con importantes amenazas potenciales a la salud humana y de los ecosistemas. La importancia de esta situación es tal que las Naciones Unidas lo ha incuido en los Objetivos de Desarrollo Sustentable como Meta No. 6 (Garantizar la disponibilidad de agua y su gestión sostenible y el saneamiento para todos). Hay una falta de información acerca de la ocurrencia de los CEs en algunas regiones del mundo, significativamente en países en vías de desarrollo como países de América Latina. La ocurrencia de CEs esta relacionada a la falta de legislación, a los altos costos de análisis en matrices ambientales y a la poca información de sus probables efectos.

La ocurrencia del NF como CEs en cuerpos de agua es difícil de evaluar, por lo tanto, es complicado determinar la fuente de contaminación. Sin embargo, la presencia de 4-NF en cuerpos de agua podría estar asociado al uso de detergentes. Tres diferentes escenarios fueron evaluados (agua recreacional, aguas residuales y agua potable). Las concentraciones de 4-NF encontradas en agua recreacional se puede deber al turismo, a comunidades lavando ropa y a descargas ilegales en ríos. La presencia de 4-NF en descargas de aguas residuales pueden estar asociadas al uso y disposición de detergentes por parte de la industria y hogares. Actualmente en México no existe regulación alguna para el uso del NF y sus etoxilados, por lo que es común encontrar a estos compuestos en todo tipo de detergentes debido a su bajo costo y propiedades surfactantes. El agua de la llave en México no es potable, por lo que se usan garrafones reciclables. Estos son lavados con detergente continuamente, por lo que un enjuague deficiente podría ser la causa de las altas concentraciones de 4-NF enontradas en las muestras analizadas. Es necesario analizar el agua potable antes de ser embotellada para poder discutir esta hipótesis. Además, los procesos de purificación convencionales no eliminan a disruptores endocrinos como el NF y en agua puede ser degradado a cadenas etoxiladas más cortas en el proceso de tratamiento y, por lo tanto, contaminar el agua. Por otro lado, el proceso de clorinación en los sistemas de suministro de agua forman subproductos como el monocloro-NF y el dicloro-NF, compuestos con actividad estrogénica.

Las siguientes limitaciones fueron consideradas al interpretar los resultados de los diferentes escenarios evaluados: 1) la cantidad limitada de muestras de agua en cada escenario y 2) las muestras recabadas son puntuales o tomadas en un tiempo y lugar específico, por lo tanto, no son representativas de cada escenario. Sin embargo, el objetivo de evaluar 4-NF en diferentes fuentes de agua fué cumplido y los resultados presentan una visión general de la situación actual y permite evaluar los niveles de exposición en esta región. La fuente real de contaminación no puede ser definida; solo es posible identificar el sitio final en donde existe el riesgo ecológico.

La evaluación de riesgos realizada por el consumo de agua potable en humanos muestra que en general no existe un riesgo por el consumo de acuerdo a los valores establecidos por la EPA (0.1 mg kg^{-1} day^{-1}). Es importante observar que esta información solo considera adultos, a pesar de que se ha reportado previamente que los infantes son mas vulnerables a los impactos hormonales causados por xenobióticos ambientales por las importantes diferencias fisiológicas como el constante incremento de peso, mayor rango de respiración y ventilación, mayor consumo de agua y comida y un desarrollo cerebral acelerado. Algunas limitaciones fueron consideradas al interpretar los resultados de este estudio: 1) Únicamente se consideró al 4-n-NF debido a su uso común como referencia por su disponibilidad comercial, sin embargo, es importante considerar que el NF es una mezcla de aproximadamente 20 isomeros para-sustituidos con diferentes cadenas alquilo ramificadas y compuestos con estructuras intermediarias entre NFE y NF que hacen a la mezcla más tóxica. Adicionalmente, se ha demostrado que los isómeros de NF tienen diferencias significativas respecto a su actividad disruptiva endocrina por lo que no es aceptable considerar a cualquier isómero como referencia general para establecer modelos, actividades, relaciones estructurales y/o evaluación de riesgos.

La factibilidad de la sustitución del NFE por APG en detergentes fue demostrada. El detergente con APG (DAPG) ofrece una menor tensión superficial mejorando la remosión de grasa y suciedad. Por lo tanto, presenta una menor CMC significando que con menos detergente se alcanza la máxima formación de micelas mejorando el proceso de limpieza y minimizando costos económicos y ambientales. Adicionalmente, DPAG presenta una menor viscosidad, los detergentes con alta viscosidad pueden dificultar el proceso de producción y cinéticamente limita la dilución en agua. También se encontraron propiedades antimicrobianas y porcentaje de remosión de impureza satisfactorio. Sin embaro, la evaluación de otros surfactantes naturales para tener un panorama general es necesario.

Actualmente, hay muy poca información con respecto a la sustitución del NFE con APG. Se espera que la tendencia hacia la sustitución del NFE incremente debido a los múltiples beneficios que ofrecen los surfactantes naturales en términos de disponibilidad, desempeño y salud ambiental.

DISCUSSION

Emerging pollutants present a new global challenge about water quality with potentially important threats to human health and ecosystems. The importance of this issue is such that the United Nations included it in the Objectives of Sustainable Development as Goal No. 6 (Ensure access to water and sanitation for all). There is a lack of information about EPs occurrence in some regions of the world, significantly in developing countries such as the Latin American countries. EPs occurrence is linked with the lack of legislation, high analysis costs in environmental matrixes, and little information about probable effects.

The occurrence of NP as EPs in water bodies is difficult to assess, therefore, it is difficult to determine the source of contamination. However, the presence of 4-NP in water bodies could be associated with the use of detergents. Three different scenarios were evaluated (recreational water, wastewater, and drinking water). Concentrations of 4-NP found in recreational water could be attributed to tourism, people washing clothes, and illegal water discharges in rivers. The presence of 4-NP in wastewater discharges could be associated with the use of detergents. Currently, in Mexico the regulation of NP and its ethoxylates is null. So it is common to find these compounds in all types of detergents (industrial and domestic) because of their low cost and surfactant properties. Tap water in Mexico is not drinkable, instead water jugs are used. The jugs are washed with any kind of detergent each time before being refilled. A poor rinse may be the cause of the high concentrations of 4-NP in the reported samples. It is necessary to analyze drinking water before bottling to discuss this hypothesis. Also, conventional water purification processes do not remove endocrine disruptors like NP and NPE in water may be degraded into shorter ethoxylated chains in the treatment process and pollute drinking water. Besides, the chlorination process in water supply systems form byproducts like monochloro-NP and dichloro-NP that have been demonstrated to present estrogenic activity.

The following limitations have been considered when interpreting the results of the different scenarios evaluated: 1) the limited number of water samples collected in each scenario and 2) the samples were punctual or taken at a specific time and place, so they are not representative of each scenario. However, the objective of assessing 4-NP in different sources of water was accomplished and results present a general overview of the current situation and allow to assess the exposure levels in this region. The real source of contamination could not be defined; it is only possible to identify the final site in which the ecologic risk is presented.

The risk assessment performed shows that, in general, there is no risk for water intake according to the values established by the EPA (0.1 mg kg-1 day-1). It is important to acknowledge that this data only considers adults, despite it has been previously reported that children are more vulnerable to hormone impacts caused by environmental xenobiotics than adults because of the physiological significant differences such as constant increase of weight, higher respiration and ventilation range, higher relative consumption of water and food and faster brain development. Some limitations were considered when interpreting the results of this study: 1) Only 4-n-NP was considered because it is commonly used as a reference due to its commercial availability but it is important to acknowledge that NP is a mixture of approximately 20 para-substituted isomers with different branched alkyl chains and with intermediate structures compounds between NPE and NP that make the mixture more toxic. Moreover, it has been demonstrated that NP isomers have relevant differences between them regarding disruptive endocrine activity so it is not acceptable to take any isomer as a general reference to establish models, activity, and structure relations or risk assessments.

The feasibility of the substitution of NPE for APG was demonstrated. Detergent with alkyl polyglucoside (DAPG) offers a lower surface tension benefiting soil removal and lower CMC, meaning that less detergent is needed to achieve the maximum formation of micelles, and therefore improving cleaning processes, and minimizing economic and environmental costs. Also, DAPG presented lower viscosity, detergents that present high viscosity may hinder the production process, and kinetically limit dilution in water. Antimicrobial properties and a satisfactory decontamination rate were also achieved. However, the evaluation of other alternatives of natural surfactants is needed.

Currently, limited information regarding detergent formulations with APG. It is nearly expected that the trend towards substitution of NPE increases because of the multiple benefits that natural surfactants offer in terms of availability, performance, and environmental health.

CONCLUSIONES GENERALES

Los resultados de la presente tesis nos permiten conocer las implicaciones ambientales y de salud por la exposición al NF ocasionado principalmente por el desecho de detergentes. A continuación, se enumeran las principales aportaciones del presente trabajo.

1. El presente trabajo destaca la falta de regulación en America Latina con respecto a los contaminantes emergentes particularmente para el nonilfenol. La falta de normativa hace que no haya vigilancia sobre la contaminación que se provoca con esta sustancia y que puede estar presente en aguas residuales, cuerpos de agua superficiales, ríos y agua potable. El desarrollo de una estrategia coordinada, integrada y colaborativa por países en América Latina para el consumo, descarga y disposición de estos compuestos es fundamental para evitar que estas sustancias contaminen los cuerpos de agua y lleguen a los seres vivos incluido el hombre. Esto podría contribuir a la reducción y prevención de impactos negativos que los contaminantes emergentes causan en el ambiente y la salud.
2. En esta investigación se desarrolló un método analítico cromatográfico utilizando micro-extracción en fase sólida, combinado con cromatografía de gases acoplado a masas para la extracción y cuantificación de 4-NF en agua. Así mismo, se demostró la factibilidad del MBTFA como agente derivatizante, ofreciendo alta sensibilidad y asegurando limites bajos de detección.
3. Se determinaron concentraciones de 4-NF en muestras de agua recreacionales, residuales y de consumo humano recolectadas en sitios turísticos, descargas industriales y agua embotellada respectivamente El estudio piloto detectó 4-NF en 84% de las muestras analizadas. Las concentraciones en aguas recreacionales, residuales y de consumo humano fueron de hasta 12.61 µg L^{-1}, 12.20 µg L^{-1} y 6.08 µg L^{-1}, respectivamente, lo que sugiere la necesidad de explorar fuentes potenciales de contaminación debido a que, de los resultados obtenidos, el 65% de las muestras presentaron concentraciones superiores al límite establecido por la Unión Europea (2 µg L^{-1}) y el 17% concentraciones superiores a lo recomendado por la EPA (6.6 µg L^{-1})., Por lo tanto, la implementación de un plan de monitoreo en países con y sin regulaciones es recomendado.

4. Se establecen datos de referencia para establecer concentraciones mínimas de exposición y se muestra una evaluación de riesgos por la exposición al NF, a través del consumo de agua potable. Se presentan dos escenarios de exposición al NF, uno para la exposición a 4-n-NF en agua potable en países donde esta sustancia es regulada (países en Europa Central y Reino Unido) y el otro para México, donde la emisión de esta sustancia en aguas residuales no es regulada. Se detectó 4-n-NF en el 45% de las muestras de agua potable recabada en bebederos públicos y agua embotellada de las cuales, el 45% presentó concentraciones superiores a la Directiva de la Calidad de Agua para el Consumo Humano de la Unión Europea. Aunque estos resultados indican que el riesgo al NF no es significativo para el consumo humano a través de agua potable, no hay un consenso entre la comunidad científica con respecto a esta situación. Por lo tanto, es importante considerar que los parámetros actuales para la evaluación de riesgos por consumo de agua son considerados para adultos y la ingesta en niños representaría un riesgo mayor.
5. Se desarrolló un detergente utilizando APG en sustitución al NFE. Se observaron propiedades similares con respecto al porcentaje de impureza removida y superiores en propiedades físico-químicas como la tensión superficial y la concentración micelar crítica, ofreciendo algunas ventajas para el mejor desempeño del detergente.
6. La implementación de estrategias a nivel mundial para promover conciencia de los impactos negativos de un disruptor endocrino es imperativo. Adicionalmente, regulaciones ambientales en países en vías de desarrollo y un monitoreo constante global es necesario para abordar la contaminación en receptores ecológicos representando efectos significantes en el ambiente y en la salud humana.

GENERAL CONCLUSIONS

Results allow us to know environmental and health implications for NP exposure caused mainly for detergent disposal. Listed below are the main contributions of this investigation.

1. This study highlights the lack of regulation in Latin America regarding emerging pollutants (i.e. NP), which results in pollution of wastewaters, effluents, rivers, and drinking water. The lack of regulations causes poor monitoring of this compound and therefore pollutes wastewaters, surface waters, and drinking water. The development of a coordinated, integrated, and collaborative strategy by countries in Latin America for the consumption, discharge, and disposition of these compounds is fundamental to minimize pollution in water bodies and biota. This could contribute to the reduction and prevention of negative impacts that emerging pollutants cause in the environment and public health.
2. In this investigation, a chromatographic analytical method was developed using HS-SPME combined with GC-MS for the extraction and quantification of 4-NP in water. The feasibility of MBTFA as a derivatizing agent offering high sensitivity ensuring the detection of low concentrations was demonstrated.
3. Concentrations of 4-NP in water samples of recreational use and human consumption as well as in wastewater discharges were found. This pilot study detected 4-NP in 84% of the samples. Concentrations in recreational water, wastewater discharges, and drinking water were up to 12.61 $\mu g\ L^{-1}$, 12.20 $\mu g\ L^{-1}$, and 6.08 $\mu g\ L^{-1}$ respectively, which suggests the need to explore potential contamination sources because results showed that 65% of the samples present higher concentrations than the established by the European Union (2 $\mu g\ L^{-1}$) and 17% of the samples showed concentrations above the recommendation of the EPA (6.6 $\mu g\ L^{-1}$)
4. This study aims to provide a reference to establish minimum exposure concentrations and to show a risk assessment from NP exposure through water intake. Two scenarios are presented, one exposure scenario of 4-n-NP in drinking water in countries with current regulations (countries from Central Europe and United Kingdom) and another scenario in countries without any regulation in this regard (Mexico). 4-n-NP was detected in 45% of water samples collected in highly touristic places and bottled

water, of which 45% presented concentrations above the Directive on the Quality of Water Intended for Human Consumption of the European Union. Although these results do not represent a significant risk for the consumption of NP through water intake there is no consensus among the scientific community about this issue, furthermore, it is important to bear that current parameters are considered for adults, and intake in children may represent a greater risk.

5. A detergent was developed using an APG in substitution of NPE. Similar properties like the decontamination rate were found. On the other hand, better properties were observed like the surface tension and critical micelle concentration, offering some advantages to improve detergent performance.
6. The implementation of strategies in the world to promote awareness of the negative impacts of endocrine disrupter is imperative. Additionally, environmental regulations in developing countries and continuous monitoring worldwide are required to address the contamination in ecological receptors that could represent significant effects on the environment and human health.

RECOMENDACIONES

Con base a lo discutido en la presente tesis se realizan las siguientes recomendaciones:

1. Se sugiere el desarrollo de estrategias coordinadas para la determinación de efectos potenciales, ocurrencia y niveles de concentración de este contaminante para promover la elaboración de regulaciones en países de América Latina.
2. La divulgación pública y científica para promover la conciencia con respecto a los impactos negativos del NF como contaminante es recomendada para minimizar y mitigar los potenciales riesgos ecológicos y de salud.
3. El análisis de mezclas de isómeros de NF es recomendado para establecer las similitudes y/o diferencias con respecto a los resultados obtenidos correpondientes al isómero 4-NF y los impactos ecológicos y de salud que esto representa.
4. Se sugiere revisar las concentraciones establecidas por la OMS con respecto a los niveles de exposición del NF a través del consumo de agua potable ya que los valores actuales únicamente están basados en un principio precautorio y no con base en información puntual con respecto a los niveles de exposición.
5. Estudios posteriores en grupos poblacionales no estudiados (niños menores de 6 semana y fetos) son necesarios para conocer los riesgos de salud considerando que este grupo es considerado más susceptible a los efectos hormonales.
6. El análisis y evaluación de otros surfactantes naturales, como el metil ester sulfonato, es recomendado para el desarrollo de alternativas tecnológicas en la formulación de detergentes.

RECOMMENDATIONS

Based on what is discussed in this thesis, the following recommendations are made:

1. Coordinated strategies are recommended to determine potential effects, occurrence, and concentration levels of this pollutant and promote the implementation of regulations in Latin American countries.
2. Scientific and public communication is important to promote awareness regarding the negative impacts of NP as a pollutant to minimize and mitigate potential environmental and health risks.
3. The analysis of isomers mixtures of NP is recommended to establish differences and/or similarities with results obtained for 4-NP.
4. Maximum exposure concentrations of NP through water intake may be reviewed because current values are based on a precautionary principle and not with punctual information.
5. Future studies in different population groups (children <6 years old and fetuses) are needed to acknowledge potential health risks considering that this population group is more susceptible to hormonal effects.
6. Analysis and assessment of other natural surfactants, like methyl ester sulfonate, is recommended to improve technological alternatives in detergent formulation.

ANEX 1 – SUPPLEMENTARY MATERIAL

TABLE 1. CONCENTRATIONS OF EMERGING POLLUTANTS IN COUNTRIES WITH AND WITHOUT REGULATION.

Country	Emerging Pollutant	Environmental Matrix	Analytic method	LOD-LOQ	Concentration (min -max)	Reference
Brasil	Amoxicillin	Rivers	LC-MS/MS	6-20	38-287.5	(Monteiro MA, 2018)
	Sulfamethoxazole			11-34	60.3-105	
	Clarithromycin			4-11	39.2	
	Cephalexin			4-12	575.5	
	Erythromycin			0.15-0.5	< LOQ	
	Azithromycin			9-27	35.9	
Brasil	Amoxicillin	Rivers	LC-MS/MS	0.14-0.46	<0.46-1284	(Locatelli et al., 2011)
	Ampicillin			0.14-0.45	<0.45	
	Cephalexin			0.19-0.64	<0.64-2422	
	Ciprofloxacin			0.13-0.41	<0.41-119	
	Norfloxacin			0.13-0.41	<0.41-51	
	Sulfamethoxazole			0.24-0.78	<0.78-106	
	Tetracycline			0.76-2.5	<2.5-11	
	Trimethoprim			0.25-0.56	<0.56-484	

TABLE 1. CONCENTRATIONS OF EMERGING POLLUTANTS IN COUNTRIES WITH AND WITHOUT REGULATION.

Brasil	Estradiol	Wastewater	LC-MS-IT-TOF	9.3-31	<LOD-<LOQ	(Brandt et al., 2013)
	Ethinylestradiol			12.4-41.3	<LOD-<LOQ	
	Bisphenol A			1.2-4	55.7-308.8	
	Sulfamethoxazole			1.5-5.1	<LOD-150.8	
	Trimethoprim			1.0-3.3	23.2-113.7	
	Bezafibrate			3.4-11.3	<LOD-249.2	
	Diclofenac			5.0-16.5	<LOD-240.2	
	Miconazole			2.6-8.7	<LOD-13.9	
Brasil	Sulfamethoxazole	Wastewater	LC-MS/MS	250-800	12500-37300	(Brenner et al., 2011)
	Trimethoprim			150-500	3650-11300	
Costa Rica	Acetominophen	Surface water	LC-MS/MS	NR-7	NR-13216	(Spongberg et al., 2011)
	Carbamazepine			NR-1	NR-82	
	Triclosan			NR-10	NR-263	
	Trimethoprim			NR-7	NR-122	
	Caffeine			NR-18	NR- 1,121,446	
	Paraxanthine			NR-8	NR-592	
	Ciprofloxacin			NR-21	NR-740	
	Norfloxacin			NR-20	NR-1744	
	Ofloxacin			NR-22	NR-335	
	Cimetidine			NR-6	NR-63	
	Clindamycin			NR-3	NR-8	
	Lincomycin			NR-1	NR-11	
	Gemfibrozil			NR-41	NR-17036	
	Clarithromycin			NR-5	NR-63	
	Diclofenac			NR-12	NR-266	
	Ibuprofen			NR-5	NR-36788	
	Indomethacin			NR-7	NR-2323	
	Ketoprofen			NR-6	NR-9808	
	Sulfadimethoxine			NR-1	NR-20	

	Sulfamethazine			NR-3	NR-1626	
	Sulfamethoxazole			NR-11	NR-56	
	Sulfathiazole			NR-4	NR-39	
	Doxycycline			NR-18	NR-73722	
	Oxytetracycline			NR-<1	NR-428	
	Tetracycline			NR-44	NR-93	
	Oxacillin			NR-63	NR-7571	

TABLE 1. CONCENTRATIONS OF EMERGING POLLUTANTS IN COUNTRIES WITH AND WITHOUT REGULATION.

Ecuador	Acesulfame	Rivers	LC-MS/MS	NR-10	0.7-31	(Voloshenko-Rossin et al., 2015)
	Carbamazepine			NR-1	11.5-830	
	Acridine			NR-1	ND-12.5	
	Acridone			NR-1	2-14	
	Caffeine			NR-2	2-5597	
	Sulphamethoxazole			NR-1	6-309	
	Venlafaxine			NR-0.1	ND-400	
	O-desmethylvenlafaxine			NR-0.1	5.5-590	
	Fluoxetine			NR-10	ND-ND	
	Cocaine			NR-1	ND-560	
	Benzoylecgonine			NR-10	ND-1065	
Mexico	Clofibric acid Salicylic acid Ibuprofen Ketoprofen Naproxen Diclofenac Gemfibrozil 2,4-dichlorophenoxiacetic acid	Groundwater	GC-MS/MS	0.5-NR 0.25-NR 0.25-NR 0.25-NR 0.25-NR 1.0-NR	ND-ND 1-464 ND-ND ND-ND ND-ND ND-ND 1 ND-ND ND-ND 1-47	(Felix-Canedo et al., 2013)

	Pentachlorophenol 4-nonylphenol Triclosan Bisphenol A Butylbenzylphthalate di-2-ethylhexylphthalate estrone 17β estradiol 17α ethynilestradiol			0.5-NR 0.5-NR 0.2-NR 1.0-NR 0.1-NR 0.5-NR 0.5-NR 0.5-NR 0.005-NR 0.005-NR 0.05-NR	1-345 1-10 1-82 19-232 ND-ND ND-ND ND-ND	
Mexico	Clofibric acid Salicylic acid Ibuprofen Ketoprofen Naproxen Diclofenac Gemfibrozil 2,4-dichlorophenoxiacetic acid Pentachlorophenol 4-nonylphenol Triclosan Bisphenol A Butylbenzylphthalate di-2-ethylhexylphthalate	Surface water	GC-MS/MS	0.5-NR 0.25-NR 0.25-NR 0.25-NR 0.25-NR 1.0-NR 0.5-NR 0.5-NR 0.2-NR 1.0-NR 0.1-NR 0.5-NR 0.5-NR	ND-ND 29-309 15-45 21-42 9-10 52-186 28.32 ND-ND ND-ND 89-655 16-19 7 5-201 75-2282 ND-ND ND-ND ND-ND	(Felix-Canedo et al., 2013)

	estrone			0.5-NR		
	17β estradiol			0.005-NR		
	17α ethynilestradiol			0.005-NR		
				0.05-NR		

TABLE 1. CONCENTRATIONS OF EMERGING POLLUTANTS IN COUNTRIES WITH AND WITHOUT REGULATION.

Mexico	Azithromycin	Wastewater	LC/ESI-MS/MS	1.8-NR	29.4-211	(Lesser et al., 2018)
	Ciprofloxacin			16.5-NR	23.7-2570	
	Clarithromycin			4.3-NR	8.42-1180	
	Cloxacillin			3.2-NR	10-10.4	
	Enrofloxacin			9.0-NR	22.8-50	
	Erythromycin			0.5-NR	25.9-1140	
	Lincomycin			3.2-NR	17.6-3710	
	Norfloxacin			50.5-NR	193	
	Ofloxacin			1.8-NR	1.82-1120	
	Oxacillin			3.1-NR	3.48	
	Sulfadiazine			2.0-NR	3.45-332	
	Sulfadimethoxine			0.4-NR	0.56-1.13	
	Sulfametazine			1.1-NR	1.21-75.5	
	Sulfamethoxazole			0.7-NR	5.2-6570	
	Sulfanilamide			8.3-NR	194-440	
	Sulfathiazole			1.6-NR	44.9-85	
	Trimethoprim			2.0-NR	19.3-1610	
	Oxytetracycline			6.0-NR	23.5-225	
	Tetracycline			6.6-NR	45.9-51.1	

TABLE 1. CONCENTRATIONS OF EMERGING POLLUTANTS IN COUNTRIES WITH AND WITHOUT REGULATION.

Mexico	Acetaminophen	Surface water	HPLC-ESI-MS/MS	2.6-28	2400-4460	(Rivera-Jaimes et al., 2018)
	Diclofenac			1.0-3.2	1100-1276	
	Ibuprofen			1.3-4.4	502-1106	
	Indomethacin			0.3-1.0	112-164	
	Ketoprofen			0.5-1.7	ND-ND	
	Mefenamic acid			0.8-2.6	ND-ND	
	Naproxen			0.2-0.8	300-4820	
	Propyphenazone			0.2-0.8	ND-ND	
	Salicylic acid			0.5-1.7	200-664	
	Chloramphenicol			0.2-0.6	ND-ND	
	Sulfamethoxazole			1.2-4.1	76-222	
	Trimethoprim			1.0-3.2	34-44	
	Glibenclamide			0.6-2.0	ND-ND	
	Cimetidine			0.5-1.6	ND-ND	
	Enalapril			0.7-2.3	ND-ND	
	Pentobarbital			10-33	ND-ND	
	Clembuterol			0.2-0.5	ND-ND	
	Salbutamol			0.9-2.9	ND-ND	
	Atenolol			9.4-31	12-16	
	Betaxolol			1.1-3.7	ND-ND	
	Carazolol			0.7-2.5	ND-ND	
	Nadolol			2.5-8.3	ND-ND	
	Pindolol			0.6-2.1	ND-ND	
	Propanolol			0.3-0.8	ND-ND	
	Sotalol			0.9-3.1	ND-ND	
	Timolo			0.3-0.9	ND-ND	
	Furosemide			0.3-0.9	ND-ND	
	Hydrochlorothiazide			1.1-3.9	ND-ND	
	Famotidine			1.1-3.7	ND-ND	
	Bezafibrate			4.7-16	1120-2100	

	Clofibric acid			0.2-0.6	ND-ND	
	Gemfibrozil			6.4-21	46-368	
	Carbamazepine			0.6-1.9	52-276	
	Diazepam			0.4-1.4	ND-ND	
	Fluoxetine			0.7-2.4	ND-ND	

TABLE 1. CONCENTRATIONS OF EMERGING POLLUTANTS IN COUNTRIES WITH AND WITHOUT REGULATION.

Mexico	Albuterol	Wastewater	HPLC-MS/MS	NR-0.59	NR-8.17	(Estrada-Arriaga et al., 2016)
	Amphetamine			NR-6.24	NR-56	
	Atenolol			NR-1.55	NR-88.2	
	Cotinine			NR-1.94	NR-42.1	
	Metformin			NR-11.2	NR-3010	
	Ranitidine			NR-0.3	NR-187	
	Triamterene			NR-0.34	NR-1.14	
	Tetracycline			NR-13.8	NR-24.1	
	Furosemide			NR-24.96	NR-53.4	
	Gemfibrozil			NR-2.9	NR-5.75	
	Glyburide			NR-5.8	NR-16.1	
	Hydrochlorothiazide			NR-18.6	NR-91	
	Ibuprofen			NR-29	NR-56.6	
	Naproxen			NR-7.59	NR-119	
	Triclocarban			NR-4.8	NR-11.6	
	Paracetamol			NR-17.1	NR-17.3	
	Azithromycin			NR-13.5	NR-82.2	
	Caffeine			NR-29.0	NR-57	
	Carbamazepine			NR-2.9	NR-244	
	Ciprofloxacin			NR-13.2	NR-203	
	Clarithromycin			NR-2.9	NR-362	
	Dehydronifedipine			NR-0.26	NR-10.8	
	Diphenhydramine			NR-1.16	NR-52	
	Diltiazem			NR-0.86	NR-6.97	
	Erythromycin-H2O			NR-4.44	NR-648	

	Fluoxetine			NR-2.9	NR-6.93	
	Lincomycin			NR-5.8	NR-183	
	Ofloxacin			NR-2.9	NR-291	
	Sulfadiazine			NR-8.34	NR-429	
	Sulfamethoxazole			NR-0.47	NR-2330	
	Trimethoprim			NR-0.9	NR-160	
	1,7-Dimethylxanthine			NR-116.0	NR-673	
	Alprazolam			NR-0.28	NR-1.51	
	Amitriptyline			NR-0.26	NR-2.36	
	Benzoylecgonine			NR-0.66	NR-32.7	
	Cocaine			NR-0.29	NR-3.31	
	N,N-diethyl-meta-toluamide			NR-1.16	NR-117	
	Desmethyldiltiazem			NR-0.29	NR-1.69	
	Diazepam			NR-0.29	NR-5.91	
	Meprobamate			NR-1.73	NR-74.1	
	Metoprolol			NR-5.9	NR-205	
	Norverapamil			NR-0.29	NR-2.27	
	Propoxyphene			NR-0.58	NR-6.89	
	Propranolol			NR-3.86	NR-53.8	
	Sertraline			NR-0.77	NR-7.38	
	Theophylline			NR-93.0	NR-571	
	Valsartan			NR-7.73	NR-238	
	Verapamil			NR-0.29	NR-3.28	
	4-Epitetracycline			NR-11.6	NR-14.4	
	Oxytetracycline			NR-10.6	NR-24.3	
	Androstenedione			NR-2.16	NR-9.56	
United States	Ciprofloxacin	Wastewater	LC-MS/MS	43-NR	200-1400	(Batt et al., 2007)
	Sulfamethoxazole			27-NR	210-2800	
	Tetracycline			52-NR	61-1100	

	Trimethoprim			68-NR	210-790	

TABLE 1. CONCENTRATIONS OF EMERGING POLLUTANTS IN COUNTRIES WITH AND WITHOUT REGULATION.

Spain	Ibuprofen	Wastewater (influent)	GC/MS	23-NR	34-168	(Gómez et al., 2007)
	Acetaminophen			32-NR	29-246	
	Caffeine			1-NR	52-192	
	1,7-Dimethylxanthine			14-NR	48-111	
	2,7/2,8-dibenzodichloro-p-dioxine			43-NR	<LOD-4.3	
	Carbamazepine 10,11-epoxide			70-NR	0.3-0.5	
	Dipyrone			45-NR	4.7-24	
	Chlorfenvinfos			21-NR	<LOD-3.7	
	Triclosan			17-NR	0.39-4.2	
	Diclofenac			100-NR	0.2-3.6	
	Bisphenol A			7-NR	0.72-3.4	
	Carbamazepine			30-NR	0.12-0.31	
	Codeine			8-NR	2.8-11	
	Permethrin			28-NR	<LOD-03	
Portugal	Azithromycin	Surface water	LC-MS/MS	Reported as ranged from 2.01 to 8.24	32.15-35.66	(Pereira, 2017)
	Ciprofloxacin				ND-ND	
	Clarithromycin				24.8-39.10	
	Erythromycin				32.89-38.80	
	Bezafibrate				11.86-15.52	
	Gemfibrozil				6.69-10.34	
	Simvastatin				ND-ND	
	Carbamazepine				<LOQ-<LOQ	
	Citalopram				20.70-52.97	
	Desmethylcitalopram				ND-ND	
	Fluoxetine				25.37-25.37	

	Norfluoxetine				ND-ND	
	Setraline				23.30-23.30	
	Desmethylsertraline				ND-ND	
	Diclofenac				25.13-51.24	
	4-Hydroxydiclofenac				ND-ND	
	Ibuprofen				ND-ND	
	Naproxen				ND-ND	
	Paracetamol				<LOQ-69.15	
	4-Aminophenol				ND-ND	
	17β-Estradiol				ND-ND	
	Estrone				ND-ND	
	17α-Ethinylestradiol				ND-ND	

TABLE 1. CONCENTRATIONS OF EMERGING POLLUTANTS IN COUNTRIES WITH AND WITHOUT REGULATION.

UK	Ibuprofen	Wastewaters	LC-MS/MS	20-NR	<20-	(Ashton et al., 2004)
	27256 Diclofenac			20-NR	<20-2349	
	Propanolol			10-NR	16-284	
	Dextropropoxyphene			20-NR	<20-585	
	Mefenamic acid			50-NR	<50-1440	
	Erythromycin			10-NR	<10-1842	
	Trimethoprim			10-NR	<10-1288	
	Acetyl-			50-NR	<50-2235	
	sulfamethoxazole			50-NR	<50-132	
	Sulfamethoxazole			10-NR	<50-42	
	Tamoxifen					
UK	Ibuprofen	Rivers	LC-MS/MS	20-NR	<20-	(Ashton et al., 2004)
	1555 Diclofenac			20-NR	<20-<20	
	Propanolol			10-NR	<10-115	
	Dextropropoxyphene			20-NR	<20-<20	
	Mefenamic acid			50-NR	<50-<50	
	Erythromycin			10-NR	<10-57	
	Trimethoprim			10-NR	<10-36	

	Acetyl-sulfamethoxazole Sulfamethoxazole Tamoxifen			50-NR 50-NR 10-NR	<50-<50 <50-<50 <10-<10	

TABLE 1. CONCENTRATIONS OF EMERGING POLLUTANTS IN COUNTRIES WITH AND WITHOUT REGULATION.

China	Sulfadiazine	Wastewater (influent)	LC-MS-ESI	Reported as ranged from 0.2 to 17.5	202.8-257.8	(Yan et al., 2014)
	Sulfamethazine				129-174.4	
	Sulfamethoxazole				2460.4-3180	
	Trimethoprim				51.9-98.8	
	Ofloxacin				276.7-401.5	
	Norfloxacin				186.3-225.1	
	Moxifloxacin				<MQL-27.6	
	Erythromycin				238.6-275.4	
	Roxithromycin				359.7-434.6	
	Azithromycin				330.27-376.5	
	Ibuprofen				243.8-296.5	
	Acetaminophen				6813.5-7515.6	
	Bezafibrate				98.75-140.13	
	Clofibric acid				17.5-47.9	
	Gemfibrozil				12.7-18.2	
	Metoprolol				43.18-54.1	
	Amlodipine				<LOQ-<LOQ	
	Atorvastatin				1.0-1.9	
	Simvastatin				101.4-133.7	
	Carbamazepine				9.8-20.1	
China	Sulfadiazine	Wastewater (disinfection effluent)	LC-MS-ESI	Reported as ranged from 0.2 to 5.6	117.8-174.8	(Yan et al., 2014)
	Sulfamethazine				31.7-47.2	
	Sulfamethoxazole				1060.3-1212.2	
	Trimethoprim				37.9-75.5	
	Ofloxacin				43.0-829	
	Norfloxacin				25.5-34.2	

Moxifloxacin	5.7-7.7
Erythromycin	135.9-174.0
Roxithromycin	300.6-386.4
Azithromycin	58-111
Ibuprofen	11.9-16.3
Diclofenac	2.9-3.7
Acetaminophen	<LOQ-10
Bezafibrate	50.2-92.3
Clofibric acid	15.4-17.1
Gemfibrozil	2.7-3.4
Metoprolol	51.1-82.5
Amlodipine	4.65.3
Atorvastatin	<LOQ-0.6
Simvastatin	<LOQ-30.2
Carbamazepine	13.6-21.0

TABLE 1. CONCENTRATIONS OF EMERGING POLLUTANTS IN COUNTRIES WITH AND WITHOUT REGULATION.

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* Units: ng L^{-1}; NR: not reported; ND: not detected; LC-MS/MS: liquid chromatography-tandem mass spectrometry; LC-MS-IT-TOF: liquid chromatography system coupled to a hybrid high resolution mass spectrometer consisting of an ion-trap and time of flight; GC-MS/MS: gas chromatography-tandem mass spectrometry; GC/MS: Gas chromatography coupled to mass spectrometry; LC/ESI-MS/MS: liquid chromatography coupled to electrospray ionization-tandem mass spectrometry; HPLC-ESI-MS/MS: High-performance liquid chromatography coupled to electrospray ionization-tandem mass spectrometry; HPLC-MS/MS: high performance liquid chromatography-tandem mass spectrometry; LC-MS-ESI: liquid chromatography mass spectrometry equipped with an electrospray ion source.

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