RESEARCH PROJECT

TITLE: Use of supramolecular solvents in the identification, detection and quantification of substances of particular concern or endocrine disruptors released from Michigan-type occlusal splints in saliva: an in vitro and in vivo pilot study.

PROMOTOR: Universidad Europea de Madrid **PRINCIPAL**

INVESTIGATOR: Susana David Fernández **CENTRE:**

Policlínica Universidad Europea de Madrid

1 INTRODUCTION

1.1 PROBLEM DEFINITION

The use of plastics in medical specialties, dentistry being no exception, has been a singular advance in the prevention of the spread of diseases. However, some of them contain substances considered endocrine disruptors that mimic or antagonise the action of endogenous hormones, alter their metabolism and synthesis, and modulate the levels of their receptors. Given their impact on health and well-being, the evolution in this respect has been remarkable: in 1993 Colborn et al. coined the concept "endocrine disruption"; Weybridge in 1996 defined endocrine disruptor (ED) as: "an exogenous substance or mixture that alters the functions of the endocrine system, causing adverse health effects in an intact organism, its progeny or (sub)populations". In 2002, the World Health Organisation (WHO) and the International Programme on Chemical Safety (IPCS) clarified what *can cause* such alterations (endocrine disrupting potential). In 1999, the European Commission published the communiqué*: Community strategy on endocrine disrupters, a series of substances suspected of interfering with the hormone systems of humans and wildlife*. On 1 June 2007, the REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) regulation comes into force to collect and evaluate information on the properties and hazards of substances. The European Chemicals Agency (ECHA) also considers substances of very high concern (SVHC). These are substances that may have serious and often irreversible effects on human health and the environment, and are listed in Annex XV of the REACH regulation. It is established at the level of

European a guideline to follow: Identify to assess and monitor. In 2019, the European Commission created a "roadmap" for the review of compliance with the "Fitness Check" for endocrine disrupters, which aims to protect health and the environment.

Numerous organisations, objectives, and laws are shaped. Among these provisions, those concerning dental devices or restorations are shown in section 10.4 of Annex 1 of the European Medical Devices Regulation (MDR) Regulation (EU) 2017/745, focusing on action on the identification of substances considered carcinogenic, mutagenic or toxic for reproduction (CMR) and/or endocrine disruptors.

In dentistry, the use of polymers, acrylics or resin-based composites (RBC) ranges from dental cements, fissure sealants, temporary acrylic materials, definitive and aesthetic restorations, partial dentures, to splints for the treatment of bruxism. Efforts are being made to develop materials that not only aesthetically but also biomechanically mimic the teeth and are easily repairable. This is why plastic materials are becoming increasingly popular in all dental specialties, from paediatric dentistry to gerontology. However, it is striking to find in their composition monomers such as bisphenol A (BPA), triethylene glycol dimethacrylate (TEGMA), methacrylate urethane, BisGMA copolymer (UDMA), or hydroxyethyl methacrylate (HEMA), substitutes for bisphenols: Bisphenol S (BPS), Bisphenol F (BPF), among others; whose purpose is to provide malleability, adhesion and strength, but which are not inert, by their nature. The possible hormonal, endocrine, neuronal, carcinogenic, mutagenic, etc. effects of the most common endocrine disruptors such as bisphenols and phthalates are well known, but even their substitutes have undesirable physiological effects. The effects of endocrine disruptors (EDs) and substances of particular concern (SVHCs) also alter dental genesis, as well as the microbiome, facilitating the proliferation of series with higher parodontogenic or cariogenic potential, favouring the two most common pathologies such as caries and periodontitis, and even malignant lesions and alterations in tooth formation (*Bisphenol A and Incisive Molar Hypomineralisation:: Topic Review - Dialnet*, n.d.; Chimenos-Küstner et al, 2019; Chimenos-Küstner & EM, n.d.; Da & Tavares, n.d.; Jacomossi, 2019; Piedrahita Sánchez et al., 2021; Tejada, 2021) .

Their dose-response curve is not monotonic, they alter biogenesis, being up to childbearing age, the window of greatest risk (Kakonyi et al., 2021). If we take into account that our activity reverts at any time in the life cycle of the population, the time that the restorations or devices remain in the mouth, the oral conditions (pH, occlusion, temperature...) that facilitate the wear and release of particles, and clinical protocols with levels of evidence that can be improved, it would be desirable to know the current status of dental materials of a plastic nature, in accordance with European regulations on identification and prevention (de Angelis et al., 2022; de Nys, D., 2022), 2022; de Nys, Duca, et al., 2021; Mulligan et al., 2022; van Landuyt et al., 2011; Vervliet et al., 2022).

1.2 BACKGROUND

Despite the fact that the dose-effect curve is not monotonic, in 2015 the European Food Safety Authority (EFSA) published a new global limit for BPA exposure and toxicity at 4 µg/kg body weight/day, compared to the 50 µg/kg/day estimated by the US Environmental Protection Agency (USEPA) in 1993 ("Hormones and Endocrine-Disrupting Chemicals: Low-Dose Effects and Nonmonotonic Dose Responses", published in March 2012 in Endocrine Reviews). On 15 December 2021, a draft proposal was made to reduce the limit by 0.04ng/kg/day, in view of the evidence shown in the literature from 2015-2018 (publication and testing of the risk assessment protocol in 2017 and EFSA https://www.efsa.europa.eu/en in 2019). With regard to BPA substitutes, it is observed that some show even more genotoxicity, such as BPF; alterations in testosterone levels such as bisphenol B (BPB); or obesogenic activity such as BPS ((Alharbi et al., 2022; Chen et al., 2023; Karrer et al., 2018; Lu et al., 2022; Moreno-Gómez-Toledano et al., 2022, vansecal II, Tolerable Dose Intake).

Despite all this, an alternative to plastic materials is not foreseen in the short to medium term, on the contrary: an increase of 6.9 to 13% of aligners is estimated in France, Italy and Germany (Koletsi et al., 2022), and the compound annual growth rate (CAGR) is estimated to be 13.5% between 2020 and 2025 (Mordor Intelligence, industries reports). This same upward trend extends to relaxation splints, made of acrylic materials, which remain in the mouth for at least 6h/day for years. These are part of the treatment of bruxism. The 'White Paper 2023: population-based survey of oral health in post-pandemic Spain 2023', shows an increase in the number of patients with bruxism.

increase from 6% in 2019, to 23% at present, in Spain ((Carrillo-Diaz et al., 2022; Dias et al., 2022; *List of Results: BRUXISM INCREASE AFTER COVID: EBSCOhost*, n.d.; Osses-Anguita et al., 2023). This is because its aetiology is closely associated with stress. One of the consequences of bruxism is the fracture of teeth, whether or not they are devitalised. As an alternative, the restoration of lost tooth tissue is indicated with inlays made of ceromers (among others). These materials fall into the group of dental composites (divided into reinforced, universal, flowable and other composites for everyday clinical use), whose global market was valued at US$ 24.7 Mn in 2021. It is estimated to grow at a CAGR of 9.0% from 2021 to 2031, and by the end of 2031, the global dental composites market is expected to reach a value of US$ 59.3 Mn. This trend extends to all fields of dentistry, due to its characteristics, as described above. The dental materials industry is also focusing on improvements in the manufacturing process of the devices (injection moulded, subtraction or milled, addition or horizontal/vertical impression) achieving a sustainable, environmentally friendly, economical practice, and releasing fewer additives (Alghazzawi, 2016; Aretxabaleta et al, 2021; Carneiro Pereira et al., 2021; de Oliveira Limírio et al., 2021; Mourouzis et al., 2020, 2022; Wei et al., 2022). In particular, 3D printing is more environmentally friendly than milling because it does not use discs, which are more costly and complex to reuse.

The main problem when studying the presence of additives released by dental materials in the organism is the extraordinary difficulty in associating them explicitly to this type of device, given that they are commonly found in our surroundings. Their multiple origins: environmental, nutritional, hygienic sources, etc., make it extremely difficult to associate the origin with the substance identified. For this reason, in vitro studies are a widely used method. However, the protocols developed to date show a certain lack of uniformity: in some, discs are analysed without considering the inhibited layer, in others their polishing does not correspond to the clinical protocol, or there is a lack of uniform extraction ratio, or the solvents are different, or salivary turnover is not taken into account (the possible alterations in its composition, pH, viscosity, or flow that may occur in the same subject, and its role in the chemical erosion of the material). On the other hand, in some of these tests, occlusal wear is not considered. They provide data on quantification and identification of the substances in saliva, mainly bisphenols, substitutes for

and phthalates, in different materials, depending on their manufacturing technique (milled, printed, injected), and their curing (thermo-, self-, photopolymerised). Clinical studies focus on paired samples, or on epidemiological, retrospective descriptive, comparative, retrospective and prospective studies where saliva, blood and urine samples are analysed to determine their assimilation. Among the methodologies used, the FDA (Food and Drug Administration) recommends High Performance Liquid Chromatography (HPLC) or Gas Chomatography (GC). Authors such as Hampe Tristan, advise Mass Spectrometry (MS), or the HPLC/MS tandem, establishing as ideal the combination of High Resolution Mass Spectrometry (HRMS) and MS, since it does not detect products such as Bis GMA, - EMA and -UDMA (De Angelis et al., 2022; Hampe et al., 2022; Kessler et al., 2020; Tichy et al., 2021). (Francisco et al., 2022; Van Landuyt et al., 2011a, 2011b; Vervliet et al., 2022; Wedekind et al., 2021).

The literature reports values within the limits (4µg/kg/day); however, some studies report higher concentrations in groups with associated pathologies than in those without. This is the case of the study carried out with patients with squamous cell carcinoma vs. control group, the results showed higher concentrations of BPA and phthalate metabolites (Hu et al., 2021; Wei et al., 2022; Zheng et al., 2017).

1.3 JUSTIFICATION

This study is framed within the European Community framework on substances that can have an impact on the health and well-being of the population, focusing on their identification, quantification, monitoring and prevention. The population susceptible to treatment of this kind is almost universal. Companies are investing in improvements in this respect, incorporating substitutes to bisphenols, with effects yet to be confirmed, generating a very dynamic and growing market. The literature demands more solid results and improvements in protocols. The Supramolecular Analytical Chemistry (SAC) research group at the University of Cordoba has developed an innovative, sustainable, specific and more economical method based on the use of supramolecular solvents (SUPRAS) combined with high performance liquid chromatography-high performance mass spectrometry (HPLC-HRMS/MS). This method allows the identification of compounds (known and unknown) by covering a wider range of substances present in the materials under study than solvents.

organic waste cannot be dealt with. Given that this method has not yet been implemented in dentistry, despite being amply tested, this pilot study has been developed to lay the foundations of a protocol, and to have a more exact and extensive knowledge of our treatments. One of the most widely used, especially after the pandemic, is the Michigan type acrylic splint, as a treatment for bruxism. It is also a removable device, which is used at least 6-8 hours a day, and does not involve the use of additional plastic materials that could be other sources of additives, such as cements, sealants or dental temporaries, as is the case with fixed prostheses. The population eligible for such treatment must have completed their bone and tooth growth (18 years of age or older). In the present study, two forms of manufacture were chosen: conventional and printed. The reason for not choosing the milled ones is because the latter generate more waste, and their mechanical response as well as the patient's preference are not better. Therefore, identifying the substances released by the acrylics, using SUPRAS and HPLC-HRMS/MS, by contrasting the in vitro and in vivo results, may shed some light on this issue.

1.4 OBJECTIVES

There is no evidence of release of substances of particular concern, or endocrine disruptors released from Michigan type acrylic occlusal splints.

General objectives: To describe and quantify the values of substances of very high concern (SVHC) or endocrine disruptors (ED) released from printed or injected acrylic Michigan type splints.

Specific objectives:

1. To identify the SEPs and EDs released by Michigan-type splints made by injection or in vitro impression technique, simulating oral environment and wear.
2. Identify SEPs and EDs in saliva released from printed or saliva-injected acrylic Michigan type splints before, during and after insertion in the mouth.
3. To quantify the levels of SEP and ED in saliva released from printed or injected acrylic Michigan type splints in saliva before, during and after insertion in the mouth.
4. Contrast the results obtained in the laboratory test with those recorded in the saliva samples of the in vivo study.

5. Relate the results to the manufacturing process, stage of exposure, time, habits and individual characteristics of each volunteer.

2 METHODOLOGY OF THE STUDY

2.1 DESIGN

This is a prospective analytical observational experimental pilot study that aims to analyse the substances of particular concern and endocrine disruptors detected in saliva, released by conventional dental devices, before and after the intervention. They will therefore be paired samples, where the control group will be the same subject, minimising the independent variables: saliva pH, microbiome, salivary flow, habits, occlusal wear, erosion by external agents and saliva.

Saliva sampling shall be carried out in accordance with the conventional protocol RD 1716/2011. The saliva containers shall comply with the requirements of the ISO 6717:2021 standard. They shall be packaged and transported according to the protocol Order JUS/1291/2010 of 13 May. All samples shall be identified with the number assigned to the volunteer, time, material and type of manufacture (processed, machined or printed), and shall be sent to the Chemical Institute for Energy and Environment (IQUEMA). University of Cordoba, where they will be analysed by the SAC group, for subsequent statistical treatment and interpretation.

2.2 LOCATION

It will take place in the facilities of the University Polyclinic of the European University of Madrid (UEM) located in Plaza de Francisco Morano s/n, registered in the Register of Health Centres of the Community of Madrid with the number CS5720.

2.3 RESPONSIBLE TEAM

2.3.1 PRINCIPAL DIRECTORS:

- Dr. Víctor Díaz-Flores García: Associate Professor in the Preclinical Department of the Faculty of Dentistry at the European University of Madrid. PI of the research group on Patient Safety in Dentistry.
- Dr. Noelia Caballero Casero: postdoctoral researcher at the Chemical Institute for Energy and Environment (IQUEMA). University of Cordoba. Member of the research group FQM-186 (Supramolecular Analytical Chemistry).
- Dr. Adriana Ivette Ávila Álvarez: Dean of the Faculty of A Coruña. Research Unit in Occupational Therapy in Non-pharmacological Interventions.

2.3.2 OPERATOR RESPONSIBLE FOR DATA AND SAMPLE COLLECTION.

- Dr. Susana David Fernández: Associate Professor in the Clinical Department of the Faculty of Dentistry at the European University of Madrid. PI of the Sustainable Dentistry and Sport research group.

2.3.3 SUPERVISOR OF COLLECTION, TREATMENT Y ANALYSIS SAMPLE

Dr. Adriana Ivette Ávila Álvarez: Dean of the Faculty of A Coruña. Research Unit in Occupational Therapy in Non-pharmacological Interventions.

- Dr. Noelia Caballero Casero: postdoctoral researcher at the Chemical Institute for Energy and Environment (IQUEMA). University of Cordoba. Member of the research group FQM-186 (Supramolecular Analytical Chemistry).
- Celia Sánchez Vallejo PhD student at the Chemical Institute for Energy and Environment (IQUEMA). University of Cordoba. And member of the research group FQM-186 (Supramolecular Analytical Chemistry).

2.3.4 STATISTICAL DATA CONTROLLER

Dr. Israel John Thuissard Vasallo (Methodological and Statistical Advisory Group, Department of Medicine, Faculty of Biomedical and Health Sciences), European University of Madrid.

2.4 POPULATION AND CALCULATION OF THE SAMPLE.

The sample will be obtained from patients who are registered in the database of the Polyclinic of the European University of Madrid, who request a Michigan type splint for the treatment of bruxism, made of acrylic, made in two of the usual ways: printed or injected, in accordance with the inclusion and exclusion criteria shown in Appendix I. As the samples were treated with an innovative methodology in the field of dentistry, such as super solvents, this was a pilot study in which two groups of 15 participants each were formed, depending on the manufacturing process of the splint: injected or printed, totaling 30 volunteers.

2.5 PERIOD OF STUDY

Recruitment is expected to be completed in January 2024, and sample collection is expected to be completed in September 2024.

2.6 DESCRIPTION OF THE STUDY

2.6.1 VOLUNTEER RECRUITMENT:

The study groups will be formed in accordance with the requirements and inclusion criteria. Each of the subjects will be informed of the purpose and methodology of the project by means of an information document for the volunteer (Appendix II) and must sign the informed consent form provided for this purpose (Appendix III); they may withdraw from participation at any time. The data of the participants will be stored in a clinical history with an individual key number, in accordance with the regulations contained in the current Organic Law 3/2018, of 5 December, on the Protection of Personal Data.

Appointments will be made as follows:

APPOINTMENT 1: A conventional computerised clinical history is taken of the patient, in which data is collected on general health, orofacial and nutritional habits and hygiene. We explain in detail what the treatment consists of: Michigan type relaxation splint (device to prevent tooth wear due to bruxism). Each intervention is governed by scientific and deontological criteria, respecting all the ethical rules of research. The following is a detailed description of the study, its duration and methodology. The four moments of saliva collection will be explained: before, during, and 3 and 6 months after placement, as follows:

-Time 0: the day of the appointment, before the intervention.

-Step 1: On the day of the appointment, device placement.

-Time 2: 3 months after device placement.

-Time 3: 6 months after treatment.

Information and informed consent will be provided.

APPOINTMENT 2: Preparation and taking of models for the Michigan type splint according to the conventional protocol for this treatment.

APPOINTMENT 3: The models will be assembled in the articulator to send and request the making of the splint, specifying whether it is injected or printed.

APPOINTMENT 4: A saliva sample is taken in the device for this purpose. After checking for proper fit and retention, the occlusal adjustments will be made with a handpiece and conventional acrylic and polishing burs. After completion of the procedure, a new saliva sample shall be taken. The samples shall be identified with the patient's assigned code and stored according to conventional standards. An appointment will be made after 6 months to continue with the study and in accordance with the check-up times for correct oral health.

APPOINTMENT 5: The intraoral check-up will be carried out, checking the intraoral health and the state of the splint, with the pertinent adjustments, according to conventional protocol. A new saliva sample will be taken. The patient will be seen after 3 months.

APPOINTMENT 6: The intraoral check-up will be carried out, checking the intraoral and splint health status, with its pertinent adjustments, according to conventional protocol. A new saliva sample will be taken. The sample collection period for this subject will end.

Prior to this, the substances released by the specific material used to make the devices will be identified in the laboratory. The reason for this is the highly dynamic nature of the dental materials market. For this purpose, the solutions will be analysed by liquid chromatography coupled to a mass spectrometer (LC-HRMS). The identification of the compounds present will be based on a non-directed suspect screening approach, comparing the results obtained in the analyses with those already existing for the

compounds included in the

list of suspects. This list shall be compiled on the basis of information gathered during the literature review, among other sources. Thus, it shall include from compounds whose presence is expected such as those indicated by the manufacturer, to emerging compounds that could be present due to storage conditions, use, etc. For the identification, statistical and chemometric techniques will be used to establish the correlation between the analytical signals obtained in the LC-HRMS and the information (e.g. masses, structure, mass spectra, etc.) available in bibliography and publicly available spectrophotometric libraries (e.g. Mass Bank). In a second phase, the compounds identified in the first stage will be prioritised according to their level of identification, frequency of detection, toxicity (if known) and availability of analytical standards. Finally, quantification of these compounds will be carried out (figure 1).

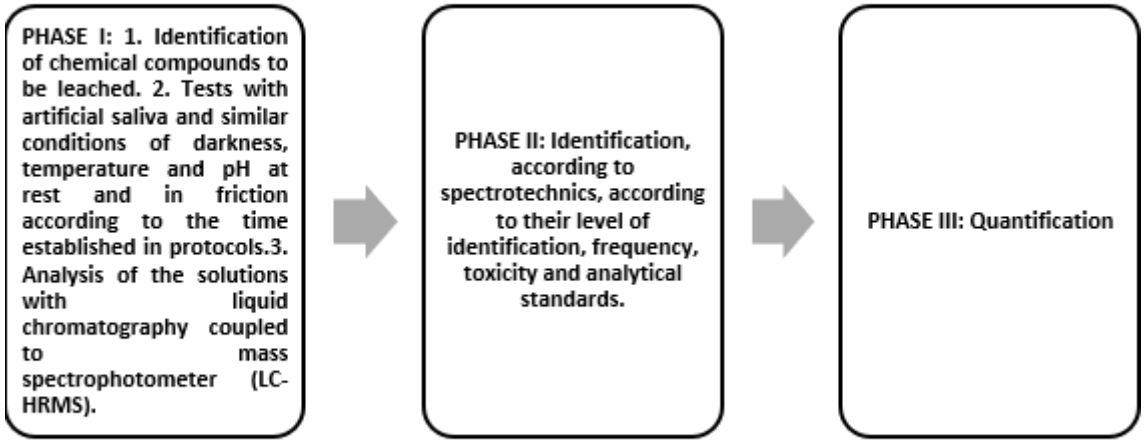

Figure 1: Timeline *In vitro* study of acrylic-based dental materials: Resting and weathering phase leaching, identification and quantification of released substances EDs and SEP. SAC innovative research group at the University of Córdoba (Spain) that belongs to the Institute of Fine Chemistry and Nanochemistry (IUNAN).

Thus, knowing the possible elements identified, the saliva samples can be treated. This is carried out with a volatile supramolecular solvent spontaneously synthesised by adding hexanol and tetrahydrofuran. The methodology to be followed is the same as previously described in the in vitro phase.

Dependent variables: substances of very high concern and endocrine disruptors. Independent variables: usual residence, type of manufacture (printed or injected), time, age, type of polymerisation.

After identification and quantification of the detected substances, the data will be collected in a database with the volunteer's information (ANNEX IV).

Finally, the results will be analysed and correlated with those obtained in the experimental laboratory phase, and the possible participating variables: age, type of material, type of manufacture and habits of the participant (figure 2).

Figure 2. Timeline of *in vivo* pilot study on substances released from injected or printed acrylic occlusal splints. University Polyclinic of the European University of Madrid.

2.7 ETHICAL AND CONFIDENTIALITY ISSUES

2.7.1 RIGHT TO BE INFORMED:

Each volunteer will be provided with an information document (Annex II), and informed consent (Annex III), which, once understood, must be voluntarily signed and returned.

2.7.2 COLLECTION AND USE OF BIOLOGICAL SAMPLES

Sample collection will be carried out in accordance with the ethical and legal requirements set out in RD 1716/2011. The containers will meet the requirements according to ISO 6717:2021, and will be packaged and transported according to the protocol Order JUS/1291/2010, of 13 May.

2.7.3 RISKS AND INCONVENIENCE FOR THE PARTICIPANT

The present study does not involve any risk for the patient, as these are procedures and treatments used in everyday dental practice, with a long clinical and scientific history. The discomforts are those inherent to these processes, without any additional discomfort.

2.7.4 ADVERSE EVENTS

It does not imply any additional consideration to those that such treatments may entail.

2.7.5 POTENTIAL BENEFITS

The benefits are those inherent to the treatments indicated: muscular improvements, avoidance of further wear and tear and more severe damage to dental tissue, loss of teeth and mucosal lesions. As an indirect benefit, knowledge of the possible substances detected will provide a better approach to the prevention of possible adverse effects.

2.8 STATISTICAL ANALYSIS

For the descriptive analysis of the variables collected, the mean ± standard deviation or median [interquartile range] will be used once the distribution has been checked, in the case of the variables collected.

quantitative variables. To describe qualitative variables, absolute (n) and relative (%) frequencies will be used and their 95% confidence interval (95% CI) will be calculated.

Chi-square tests (or Fisher's exact tests) will be used to study differences between qualitative variables. In case of quantitative variables, Student's t-test or Man-Whitney U-test will be used, depending on whether they follow parametric or non-parametric behaviour. Binary logistic regression models will be used to determine the Odds ratio (OR) of seroprevalence and its 95% confidence interval (95% CI) to analyse the effect of each variable on the results obtained.

All statistical calculations were performed using SPSS statistical software (version 25.0 IBM Corp.; USA). Statistically significant differences were assumed to exist if the p-value obtained was less than or equal to 0.05.

2.9 DISSEMINATION OF RESULTS

The members of the study's research team are committed to disseminating the results in scientific media, supports and publications.

2.10 EXPENSES AND FINANCIAL COMPENSATION

This study is exempt from financial compensation to participants.

ANNEX I

INCLUSION CRITERIA FOR THE FORMATION OF THE "MICHIGAN SPLINTS" GROUP

- Be of legal age
- To be able to attend Polyclinic for 6 months
- Require a Michigan type acrylic relaxation splint.
- Not pregnant
- Not be breastfeeding

EXCLUSION CRITERIA:

- Being a minor
- Allergy to any type of plastic material, acrylic or resins used in dentistry.

ANNEX II

VOLUNTEER INFORMATION SHEET FORMAT

Information document for volunteers on disruptors and substances of particular concern in critical materials in dentistry.

Study Name: Use of supramolecular solvents in the identification, detection and quantification of substances of very high concern and endocrine disruptors released from Michigan-type occlusal splints in saliva: an in vitro and in vivo pilot study.

Principal Investigator:Dr. Susana David Fernández

Place of research: University Polyclinic of the Universidad Europea. Plaza Francisco Morano s/n. 28005. Madrid. Spain (registered in the Registry of the Community of Madrid under No. CS5720).

OBJECTIVE OF THE STUDY:

To analyse the presence of additives in saliva before, during and after common clinical procedures in dentistry.

STUDY PROCEDURE:

This study will be carried out in accordance with the Code of Ethics and Professional Deontology drawn up by the Ethics and Deontology Commissions of the Spanish Dental and Stomatology Associations.

The conventional protocol will be carried out (clinical history, treatment plan, budget and consents) that any dental treatment entails. Once you have decided to start your dental treatment, on the day your splint is to be given to you, saliva samples will be taken, explaining the procedure at the previous appointment. Subsequently, you will be given an appointment every 3 months to take a saliva sample, according to the detailed explanations that you will be given beforehand. The study lasts 9 months from the first appointment to the last.

All the data obtained from the volunteers will be stored in a medical record with an individual key number that will be filed in a space designated for this purpose, in the University Polyclinic of the Universidad Europea following the following regulations

contained in the current Organic Law 3/2018, of 5 December, on the Protection of Personal Data.

REVIEW

Volunteers will be notified of successive scheduled appointments (4 times in total).

RISK TO THE PARTICIPANT:

All the materials used in this study follow the sterilisation and disinfection procedure contained in ORDER 288/2010, of 28 May, which regulates the general and specific technical requirements for health centres and services without hospitalisation, and for health services integrated in non-health organisations in the Community of Madrid.

As this is a non-invasive test in which only saliva samples are taken, there is no risk to the volunteer.

The examiner has more than 25 years of clinical experience.

BENEFITS:

There is no direct benefit to participating in this study as a volunteer.

ALTERNATIVES:

As this is a non-invasive observational study, there is no alternative to it.

VOLUNTARY PARTICIPATION AND WAIVER

Your participation in this study is voluntary. You may withdraw from the study at any time during the study.

Investigators may terminate their participation in the study for any of the following reasons:

1. Development of medical conditions that prevent you from participating in the study.
2. If, during the study, the prosthesis is damaged or lost in such a way that it cannot be followed up.
3. If you become pregnant between the recruitment period and the start of the study.
4. If you fail to keep scheduled appointments
5. If the study is cancelled.

PARTICIPATION RIGHTS/AVAILABILITY OF INFORMATION

If you have any questions before or during the study contact the principal investigator (Dr. Susana David Fernández, 913858800, susana.david@universidadeuropea.es)

ANNEX III

INFORMED CONSENT

Informed Consent to Participate in a Clinical Study on Disruptors and Substances of Very High Concern in Acrylics in Dentistry.

Study Name: Use of supramolecular solvents in the identification, detection and quantification of substances of very high concern and endocrine disruptors released from Michigan-type occlusal splints in saliva: an in vitro and in vivo pilot study.

Project Code: 23.282

Principal Investigator; Dr. Susana David Fernández

Place of research: Polyclinic of the Universidad Europea. Plaza Francisco Morano s/n. 28005. Madrid. Spain (registered in the Registry of the Community of Madrid under No. CS5720).

INTRODUCTION:

You and 29 other volunteers, who are patients of the European University, have been invited to participate in this research study on the presence of additives in dental materials of recognised quality and guarantees, used daily in dentistry. Please review the contents of this informed consent form and make sure that all your questions are answered before you decide to participate in this study. The investigators will explain all aspects of the study to you and will be available to answer any questions you may have throughout the duration of the study.

CONFIDENTIALITY CLAUSE:

The data obtained by participating in this study will be processed in accordance with Organic Law 3/2018, of 5 December, on the Protection of Personal Data, with the participating volunteer reserving the right to oppose, access, rectify or delete the data.

cancellation of your data in accordance with the procedure described in the Regulations for the development of Organic Law 3/2018, of 5 December, on the protection of personal data.

The data may be used for statistical and academic purposes, while respecting the confidentiality of the data and the personal image of the volunteer. Any publication or presentation in which the data obtained may be used will not identify the volunteer.

PARTICIPATION RIGHTS/AVAILABILITY OF INFORMATION

If you have any questions before or during the study contact the principal investigator (Dr. Susana David Fernández, 913858800, susana.david@universidadeuropea.es).

CONSENT TO PARTICIPATE:

By signing this consent form, I declare that I am 18 years of age or older. I confirm that I have read this informed consent, and that I have been given sufficient opportunity to ask any questions about this consent or about the study. I also confirm that I understand the purpose of my participation in this study, and that all my questions have been answered satisfactorily. I sign this consent form voluntarily, agreeing to participate in this study. I understand that I am not giving up any of my rights by signing this consent or participating in this study and that I will receive a copy of this consent.

_____ _____

Name, surname, ID number and signature of volunteer Date

ANNEX IV

Voluntary code	CONCENTRATIONS	CONCENTRATIONS	CONCENTRATIONS	CONCENTRATIONS
SUBSTANCES	**M .Saliva 0**	**M. Saliva 1**	**M. Saliva 2**	**M. Saliva 3**

Name, surname, ID number and signature of the researcher

Date

Sample collection table for each volunteer

VARIABLES	Volunteer 1	Volunteer 2	Volunteer 3	Volunteer 4	Volunteer 5
AGE	-				
GENDER (MALE, FEMALE, OTHER)					
MATERIAL (ACRYLIC)					
MANUFACTURING(INJECTED or PRINTED)		-			
USUAL RESIDENCE (URBAN, RURAL)			-		
EXPOSURE TIME (HOURS) BRUXIST				-	
(YES/NO)					
REFLUX (YES/NO)					
ANTIDEPRESSANTS					
(YES/NO) ANTIHISTAMINES					
(YES/NO)					
DECONGESTANTS (YES/NO)					

Variable collection table

BIBLIOGRAPHY

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