Occupational exposure to hairdressing products and thyroid disrupting effects : protocol for an exploratory field study in French hairdressers

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Abstract

Background: Cosmetic legislation is ensuring the safety of cosmetics for consumers but not for professional use. All around the world, the hairdressing sector constitutes a major occupational group including about 90% of women continuously exposed to numerous chemical composing the hairdressing products, among them the endocrine disrupting compounds such as resorcinol, parabens, phtalates, UV filters. The thyroid-disrupting chemicals are substances of concern as the incidence of thyroid dysfunction has increased recently worldwide and the long-term consequences at neurodevelopmental level in offspring after in-utero expoure were highlighted. Few biomonitoring studies explored occupational exposure to endocrine disruptors in hairdressers and up to date we found no data about the impact on thyroid hormones system.

Objective: The present study aimed to assess the occupational exposure to resorcinol in French hairdressers and to analyze the relationship with biological thyroid parameters, taking into account the occupational exposure to other thyroid disruptors (parabens, UV filters).

Methods: A cross-sectional study is carried out in 300 female hairdressers aged 18-45 years compared to 150 controls, who are recruited by six occupational health services. The hairdressers are followed during a 5-day working week to assess exposure data at individual and salon levels (urinary thyroid disruptors (resorcinol, parabens, benzophenone-3, ethylhexyl methoxycinnamate's metabolits) at different time points, daily job tasks and use of hair products, salon inventory of hairdressing and other chemical products) and blood thyroid parameters (T3, T4, TSH, thyroglobulin, thyroperoxidase and throglobulin antibodies). Information on sociodempographics and residence, alimentary and tobacco consumption, personal use of care and household products, professional career and medical history was collected by questionnaire.

Results: The inclusion of study population was planned between 2023 and 2025. Until now, a total of XX subjects (XX hairdressers belonging to XX salons, XX unexposed) were included by XX occupational health services. Since the inclusion rates of hair salons (around 10%) and of hairdressers (around 50%), are below those estimated, the inclusion period will be extended till 2026. Further, new partnership with more occupational health services are under study.

Conclusions: This study will provide new evidence on the occupational exposure of hairdressers to resorcinol and to other thyroid disruptors. The results represent the first data on occupational exposure to resorcinol in France and the relationship with thyroid hormones in hairdressers, a susceptible population of childbearing age. We proposed a timely research based on a partnership with occupational health professionals and designed following a multidisciplinary approach including biometrology and epidemiology. The collection of exposure data at both hairdressers and salon levels allow in-depth exposure assessment to the thyroid disruptors in workplace. Together with the inventory of hairdressing and other chemical products, these results may strengthen the tools for the chemical risk diagnosis and prevention in hair salons.

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Occupational exposure to resorcinol and thyroid disrupting effects: protocol for an exploratory field study in French hairdressers

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Abstract

Background: All around the world, the hairdressing sector constitutes a major occupational group including about 90% of women, most of them of reproductive age. Haidressers are continuously exposed to numerous chemicals used in hair products, among them the endocrine - disrupting compounds such as resorcinol, parabens, phthalates, UV filters. Few biomonitoring studies explored occupational exposure to endocrine disruptors in hairdressers and up to date we found no data about the impact on thyroid hormones system. Resorcinol is an oxydative hair dye with thyroid-disrupting properties that decrease thyroid hormones synthesis and could alter neurodevelopmental functions during fœtal and perinatal stages in case of maternal exposure. **Objective**: The present study aimes to assess the occupational exposure to resorcinol in French hairdressers and to analyze the relationship with biological thyroid parameters, taking into account the occupational exposure to other potential thyroid disruptors (parabens, UV filters like benzophenone and cinnamates).

Methods: A cross-sectional study is currently ongoing in female hairdressers aged 18-45 years compared to occupationally unexposed controls, who are recruited by 15 occupational health centers scattered throughout French regions. The hairdressers are followed during a 5-day working week to assess exposure data at individual level and at salon level. Urinary samples for the measurement of thyroid disruptors (resorcinol, parabens, ethylhexyl methoxycinnamate's metabolites, and benzophenone-3) are collected at different time points during workday and working week. Daily work tasks and use of hair products were self-reported within the workplace, and a complete inventory of hairdressing and chemical products within the salon is carried out. Blood thyroid

parameters are measured once and consists in thyroid hormones - triiodothyronine (T3) and tyroxine (T4), thyroid-stimulating hormone TSH, thyroglobulin, thyroperoxidase and thyroglobulin antibodies. To assess non-occupational exposure to thyroid disruptors and other confounding factors, information on sociodemographic data and place of residence, food and tobacco consumption, personal use of care products, professional career and medical history is collected by questionnaire.

Conclusions: The results will represent the first data on occupational exposure to resorcinol in France and the relationship with thyroid hormones in hairdressers, a susceptible population of childbearing age. This timely research based on a partnership with occupational health professionals is designed following a multidisciplinary approach including biometrology and epidemiology. The collection of exposure data at both hairdressers and salon levels allows in-depth exposure assessment to the thyroid disruptors in workplace. Together with the inventory of hairdressing and other chemical products, these results may strengthen the tools for the chemical risk diagnosis and prevention in hair salons.

Keywords: resorcinol; hairdresser; endocrine-disrupting chemicals; occupational exposure; thyroid gland; biomonitoring; cosmetics; hair products; thyroid disruptors; disruptors of thyroid hormones

Introduction

1) *Principles of cosmetic product regulation in the European Union: highlight on endocrine-disrupting chemicals*

In the European Union (EU), the legal framework for cosmetic products relies on the EU Cosmetic Products Regulation No. 1223/2009 [1]. The main purpose of the cosmetic products legislation is ensuring the safety of cosmetics for consumers through rigorous science-based regulation. More thoroughly, the ingredients regulation is « ingredients risk-based » accomplished by the establishment of positive and negative lists of ingredients [2] and the Scientific Committee on Consumer Safety (SCCS) provides guidance about the safety of cosmetic ingredients for consumers only, but not for professional users.

In the EU, there is no specific management of endocrine-disrupting chemicals (EDCs) in cosmetics. These substances, defined by the World Health Organization as « e*xogenous substances or mixtures that alter the function(s) of the endocrine system and consequently cause adverse effects in an intact organism, or its progeny, or (sub)populations* » [3], are evaluated and regulated on a compound-by-compound basis by the SCCS. A number of restrictions can be applied when the substances are also classified as carcinogenic, mutagenic or toxic for reproduction (CMR), or considered as substance of very high concern (SVHC, defined as substances that may have serious and often irreversible effects on human health and the environment). The EU recently introduced new hazard classes to reinforce the classification, labelling and packaging of substances and mixtures (CLP) regulation, as the central piece of the chemicals legislation among endocrine

disruptors for human health and the environment [4]. Thus, two hazard classes and corresponding statements for endocrine disruptors have been created based on their scientific strength of evidence: known or presumed endocrine disruptors (category 1) and suspected endocrine disruptors (category 2), for both human health and the environment.

Within the EU, six national competent authorities compile information about the current status of substances identified as EDCs or under evaluation for endocrine-disrupting properties within three lists, updated at least bi-annually and held on a dedicated website [5]. The three Endocrine Disruptor lists (ED lists) consist in List I: substances identified as endocrine disruptors that have undergone the full evaluation of endocrine-disrupting properties ; List II: substances that are currently under evaluation; List III: substances considered as endocrine disruptors at the national level in one of the participating Member States.

2) *EDCs in hair products: focus on thyroid hormones system - disrupting chemicals*

Cosmetic hair products belong to a wide spectrum of products ranging from shampoo, conditioner, hair dyes, to fragrances, solutions or sprays for hair bleaching, perming, straightening, styling, modeling. The endocrine disruptors in hair products are compounds that possess various functions (preservatives, hair dyeing, perfuming, UV protection…), and the most common reported in the literature are parabens, phenols, bisphenols, phthalates, cinnamates, benzyl salicylate [6-8].

a. Brief inventory of EDCs in professional hair products

We conducted in 2020 a brief inventory of EDCs identified among the compounds of 1,157 hair cosmetic products on sale to hairdressers on professionnal websites [9].

In this inventory, the content of hair products revealed the presence of resorcinol in 40% of hair dyes, parabens in around 20% of styling waxes as well as gels and lacquers for men, salicylic acid in 20% of shampoos, cyclopentasiloxane in 15% of hair care products and conditioners, butylated hydroxytoluene in 10% of hair gels or sprays. The most frequently used UV filters and absorbers were benzyl-salicylate (25% of hair products), benzophenones (15-20% of shampoos for coloured hair and styling products) and ethyhexyl-methocynnamate (10-12% of hair gels, lacquers, sprays, hair care and conditioners).

b. Thyroid hormones system and thyroid dysfunction

Thyroid hormones are involved in numerous physiological processes as regulators of metabolism, bone remodeling, cardiac function, fetal neurodevelopment and mental status [10, 11]. Thyroid function is regulated through the hypothalamus-pituitary-thyroid axis in which the hypothalamus releases thyrotropin-releasing hormone (TRH) to stimulate the anterior pituitary gland to produce

thyroid-stimulating hormone (TSH), which in turn stimulates the production and secretion of thyroid hormones (TH: tyroxine (T4) and triiodothyronin (T3)) from the thyroglobulin (Tg) stored in the thyroid follicles

The incidence of thyroid dysfunction has recently increased worldwide, particularly among women, since the ratio of thyroid dysfunction among women of reproductive age compared to men is 4:1 [12]. Hypothyroidism is the most frequent, followed by hyperthyroidism that was also associated with 4.5 fold increased risk of thyroid cancer [13]. Overall, 5–9% of adults have subclinical thyroid disease and 0.8–7.5% overt clinical thyroid disease [12, 14]. Due to the serious health consequences and financial burden associated with thyroid diseases, the identification of risk factors, especially those modifiable, is of utmost public health interest. There is compelling evidence from in vivo, in vitro and epidemiological studies that a growing number of chemicals can disrupt the thyroid function and cause adverse health effects [15, 16]. Thyroid-disrupting health effects of exposure to care and other consumer products were reported and their continuous consumption nowadays negatively affects susceptible populations, as pregnant women and their offspring, with long term adverse consequences such as atention/deficit/hyperactivity disorders, autism spectrum disorders and development delay in the offspring [17].

c. Thyroid-disrupting chemicals in hair products: focus on resorcinol

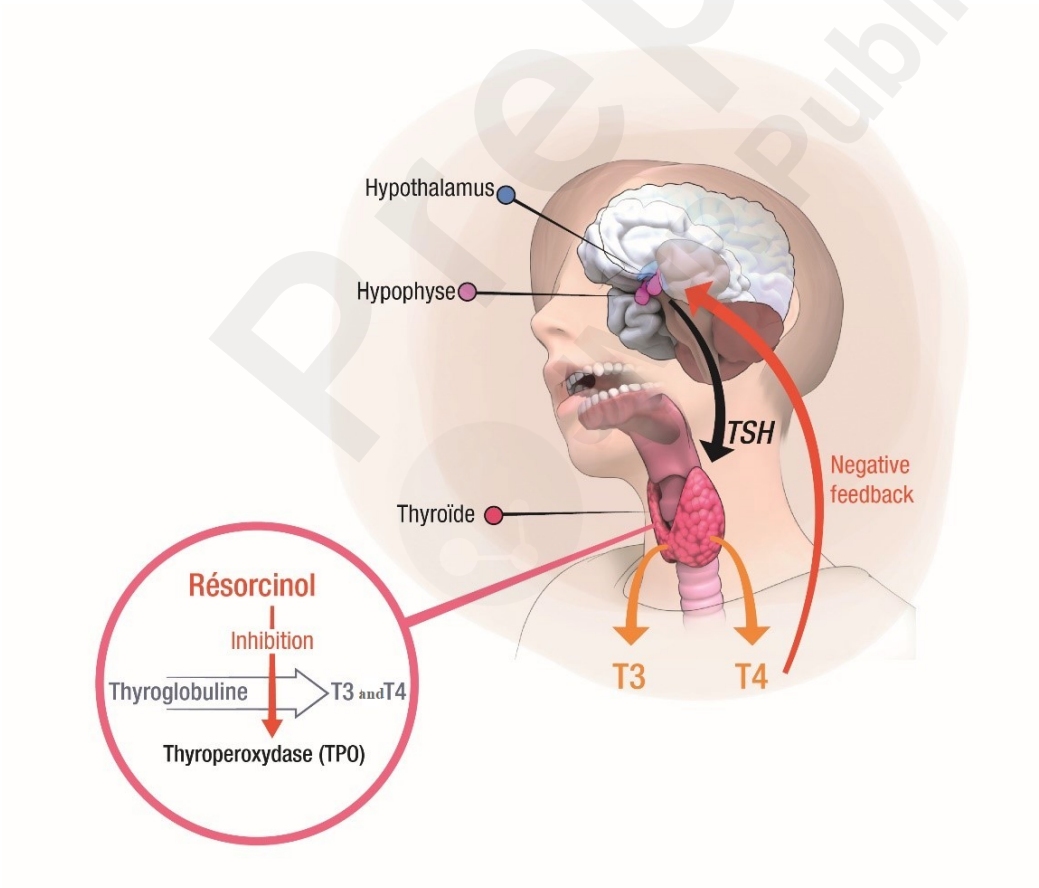
Among the compounds of hair products, parabens have been shown to have a disruptive effect on the thyroid in both experimental and human studies [16, 18], whereas for benzophenone UV filters, the relevance of human exposure needs to be studied further [19]. As regards cinnamate UV filters, only experimental studies have indicated interferences with thyroid function [8]. Resorcinol is an oxydative hair dye (maximum allowed concentration up to 1.25%) and also an ingredient in hair lotions and shampoos (maximum allowed concentration up to 0.5%) [20]. Otherwise, resorcinol is used in other industrial fields for its properties (antioxydant, antiseptic, enhancing rubber and wood products strength, UV protection, flame retardant, colorant, etc), and it is found in the environment as a result of anthropogenic activities and as a component of tobacco smoke. The global resorcinol market stood at nearly seventy thousand tons in 2022 and is expected to grow at a compound annual growth rate of 3.94 percent over the forecast period until 2032 [21].

In the professional use of resorcinol or resorcinol-containing products, the main route of exposure is skin contact since resorcinol is a molecule of low volatility. Nevertheless, inhalation may occur if resorcinol is aerosolized, as well as also oral exposure through hand-to-mouth contact.

In 2020, The French Agency for Food, Environmental and Occupational Health & Safety (ANSES) submitted a proposal for identifying resorcinol as a substance of very high concern (SVHC) on the

basis of the criteria set out in the European regulation REACH (for Registration, Evaluation, Authorisation and Restriction of Chemicals). This report was based on the scientific evidence of probable serious effects to human health in relation to its thyroid-disrupting potential shown in numerous *in vitro* and *in vivo* studies [22]. Resorcinol acts principally via the inhibition in a dose-dependent manner of the key enzyme of thyroid hormones synthesis thyroperoxidase (TPO), resulting in the blockage of thyroid hormone synthesis and decreased serum T4. Consistenly with TPO inhibition, resorcinol inhibits intrathyroid iodine uptake and organification in humans, rodents and recently fishes and lizards, leading to clinical hypothyroidism [22, 23]. Low TH levels trigger a negative feedback mechanism mediated by the hypothalamic thyrotropin-releasing hormone that stimulates the synthesis and release of TSH in the pituitary gland to compensate for insufficient levels of thyroid hormones (Figure 1). Compensatory mechanisms within thyroid gland in response to the increase in TSH (thyroid hyperplasia, thyroglobuline depletion, increasing iodine absorbtion) are not able to restore a normal thyroid function because of the direct inhibition of TPO.

Figure 1. Thyroid-disrupting mechanism of resorcinol involving TPO inhibition leading to impaired iodination of thyroglobulin in the thyroid gland, reduced thyroid hormones (T3 and T4) synthesis, and compensatory increasing in TSH level.



Since resorcinol has been used as a dermally applied ointment to treat mainly skin ulcers and more

rarely acne, clinical hypothyroidism was reported in several human cases after repeated application, as well as the reversal of symptoms and of goitre after cessation of the exposure [22]. In animals, effects on thyroid were consistent with human effects, together with histopathological changes in the thyroid and probable reproductive and neurodevelopment toxicity. For humans, the relationship between inhibition of TPO, decrease in maternal circulating thyroid hormones, and pre- and post-natal neurodevelopmental alteration in offspring is considered to be established with a high level of evidence. Lastly, even if the resorcinol is a chemical with clear thyroid-disrupting properties in humans, its thyroid disrupting effects have not been explored in the context of occupational exposure.

3) Hairdressing, occupational exposure to endocrine-disrupting chemicals and health endpoints

Around the world, the hairdressing sector constitutes a major occupational group employing a majority of women, many of whom of reproductive age [24]. In France, in 2022, there were 180,000 hairdressers, and about 90% of them were women [25]. This occupational group is continously exposed to numerous chemical ingredients composing the hairdressing products, as well as to cleaning products at the workplace [26]. In contrast to most customers using cosmetics only for a few minutes each day, hairdressers may be exposed to them for up to eight hours per day, five or six days per week, throughout their careers. The average frequency of use by consumers therefore severely underestimates the exposure of hairdressers to daily skin contact with hairdressing products [27], and the safety assessment for consumer's use differs from those for professional use. For instance, in a SCCS Report in Netherlands undertaken on 56 hair dyes, more than 37% of the products were considered as safe for consumers but exhibited margins of safety considered as risk products for professionals [28]. The routes of exposure differ following the type and use of the products. For example, during the hair coloring stages, powder may be inhaled due to mixing, skin contact may occur when the mixtures are applied, and gases or vapor could be inhaled due to evaporation [29]. Workplace characteristics (ventilation, size of salon), working technique (haircutting after applying product mixtures, use of protective equipment), hairdressers' information and prevention education are also relevant for the exposure of hairdressers [29].

The occupational hazards of cutaneous and respiratory allergic disease in hairdressing are well described, but differences were reported between information declarated in surveys and work observations, particularly for the wearing of gloves, suggesting their use not systematically during the steps of hair dyeing following application, such as washing/rinsing and haircutting [29]. Resorcinol was therefore found in most handwash samples from hairdressers after the application of hair dye, as well as after the haircut that follows hair dye, after gloves have been removed [30, 31].

Overall, the inappropriate use of gloves during tasks (sharing and reusing, wearing jewelry, unsuitable cheap gloves), inadequate skin protection, working in wet environments likely present to a large extent in hairdressers, impaired epidermal barrier function, aggregated exposure (exposure to the same ingredient via different products) among others, facilitate the penetration of hazardous substances into the skin with consequently higher risk of local and possibly systemic adverse effects [27].Recent studies assessinging chemical exposure among hairdressers are limited and mainly addressed on air contaminants in hairdressing salons such as volatile organic compounds, phthalates or particulate matter [24, 32]. Furthermore, there are few urinary biomonitoring studies that enable an evaluation of overall exposure, including the cutaneous route of exposure to hair products. [24, 33]. Few biomonitoring studies explored occupational exposure to endocrine disruptors in hairdressers [24]. In a Finnish study aimed at quantifying environmental and occupational exposure to resorcinol in urine, no difference was observed between hairdressers and non-occupationnally exposed women [34]. Nevertheless, concentration levels of resorcinol were higher in post-shift samples compared to the morning sample after at least one day off from working. In another study, urinary phthalate biomarker levels were higher in Slovakian hairdresser apprentices compared to controls. The latter were associated with negative outcomes in pulmonary function [35]. More recently, Kolena *et al*. published a second Slovakian study that confirmed significant exposure to phthalates related to hairdressing among apprentice hairdressers and found a relationship between lung function tests and anthropometric parameters adjusted for occupational exposure to phthalates [36]. The authors suggested that exposure to phthalates could affect the body composition related to fat and fat-free mass index, which could secondarily affect lung function. In comparison to controls, US hairdressers serving an ethnically diverse clientele had concentrations of urinary phthalate metabolites 10 times higher after shift [37]. These hairdressers were also exposed to 24 additionnal chemicals with median peak areas twice as high including methylparaben, ethylparaben, propylparaben, and 2-naphthol [38].

As hairdressing workforce includes a large majority of women of childbearing age, most of research focused on reproductive effets. Chemicals in hair products changed significantly over time, but an updated review including epidemiological studies published in the last two decades still reported scarce adverse reproductive outcomes (poor neonatal health indicators and perinatal adverse events) [39]. A recent Brazilian study pointed out a higher number of premature deaths due to reproductive system cancers in female hairdressers [40]. To note, the association of hairdressing occupation with cancer and reproductive diseases was consistentlty reported [26, 39], leading the International Agency for Research on Cancer (IARC) in 2010 to classify the occupational exposure of hairdressers

as probably carcinogenic (IARC group 2A).

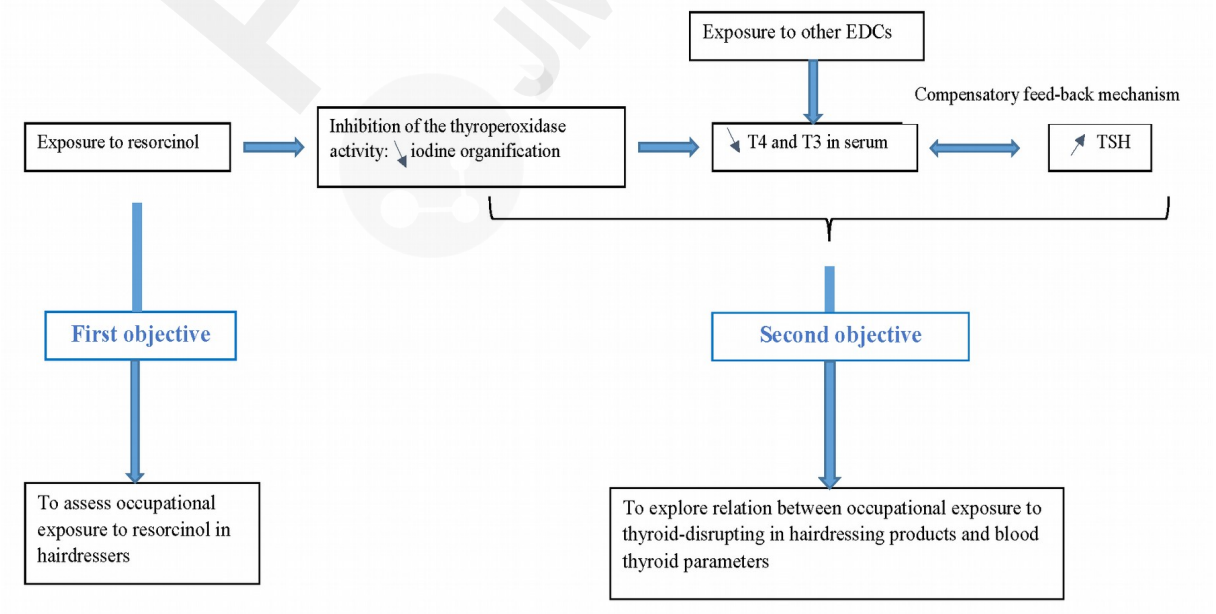
In summary, the state of the art on occupational exposure to endocrine disruptors in hairdressers highlights the small number of recent studies, the rare reports of type and magnitude of exposure, the mixture effects of all pollutants, the limitations in establishing the respective contributions of various chemicals and the causal relationships that do not enable to properly assess endocrine-disrupting effects of occupational exposures. Conducting epidemiological studies on the subject is challenging, since endocrine disruptors are ubiquitous compounds nowadays, the health effects could be delayed, mixtures need to be considered to assess endocrine disruption of ingredients into the final product, a low enough magnitude of exposure as dermal exposure to cosmetics seems not as hazardeous as dietary intake [6]. In an attempt to address these gaps in the existing literature and to increase current knowledge regarding thyroid disrupting substances in this understudied population, we proposed an exploratory field study in French hairdressers to investigate occupational exposures to the main thyroid disruptors in hair products and the relationship with blood thyroid parameters.

Study objectives

On the basis of the presumed physiopathological mechanism presented in Figure 2, this study aims to address two objectives:

- the first objective is to assess the occupational exposure to resorcinol, based on urinary measurements in hairdressers working in hair salons ;
- the second objective is to explore the association between occupational exposure to resorcinol in hairdressers and biological thyroid parameters, taking into account the occupational exposure to other endocrine disruptors like parabens and UV filters.

Figure 2. Thyroid-disrupting mode of action of resorcinol and subsequent study objectives



Methods

Study design and protocol

We designed an exposed/unexposed cross-sectional study where hairdressers are followed-up during a 5-day working week after at least one day rest. During the working week, urines samples are collected before day 1 shift, before and after day 3 and day 4 shifts, and before day 5 shift (Table 1). Daily tasks are self-reported by each hairdresser during the working week (daily work sheet) and a visit of the hairdressing salon is performed by the research team. For unexposed women, only two urinary samples are collected, at the beginning and at the end of the working week.

At the inclusion medical visit, a blood sample is collected, a medical questionnaire is administered and a self-administered questionnaire is given for all subjects. At the end of the follow-up, all daily work sheets and the self-questionnaire are collected by the research team.

Table 1. Study design and data collection time points for exposed (E) and unexposed (U) subjects.

Inclusion visit (within 14 days before the follow-up week)		**5-day working week follow-up (after at least 24 h off work)**									
Health assessment		**Exposure assessment**									
Medical questionnaire	**E/U**		**Day 1**		**Day 2**	**Day 3**		**Day 4**		**Day 5**	
Blood sampling (8 a.m.-12 a.m.)	**E/U**		**Pre-shift**	**Post-shift**		**Pre-shift**	**Post-shift**	**Pre-shift**	**Post-shift**	**Pre-shift**	
Self-questionnaire	**E/U**										
		Daily work sheet	**E**		**E**	**E**		**E**			
		Urine sampling	**E/U**			**E**	**E**	**E**	**E**	**E/U**	
		Hair salon visit	**E**								

Study population

Inclusion and non-inclusion criteria: exposed population consists in female hairdressers aged 18 to 45 years, not being an apprentice, working in a traditional hairdressing salon and having a seniority of at least one year. Home-dressing and salons with other concomittant activities (nail salon, beauty salon, etc) practised in the same room are not included. Unexposed population consists in female

employees with an office activity like secretary, computer specialist, accounting, etc. Non-inclusion criteria for both groups are history of thyroid disorders, ongoing or past pregnancy for less than 12 months, current breastfeeding, history of severe or chronic disease. All participants are asked about the date of the last personal hair dyeing (not allowed for less than 7 days before nor during the working week follow-up) and the use of iodine products for medical purposes (imaging or dermal products not allowed for less than one month before inclusion nor during the working week follow-up). In the case of recent personal hair dyeing or use of iodine products, the inclusion should be postponed.

Recruitment and inclusion of participants: The recruitment of the study population is carried out by occupational health professionals (OHP) belonging to occupational health centers spread across different French regions. Currently, fifteen occupational health centers (six from the beginning of the study and nine additional services since 2025) are voluntary to take part in the study based on their interest in endocrine-disruptor chemicals and work. The recruitment of the hairdressers is carried out by the occupational health centers on the basis of a three-step procedure: 1) identification in the medical records of the eligible hairdressers complying with the inclusion criteria of age, professional activity and medical history; 2) identification of the salons where eligible hairdressers are working to obtain a verbal approval of the salon manager to participate in the research; 3) in the case of the approval of the manager, hairdressers are informed about, then asked on the volunteering to participate in the study. Voluntary hairdressers are invited to attend the medical inclusion visit within the occupational health centers. In the case of refusal to participating, non-identifying data on the reasons of the refusal are collected if possible from the salon's manager and/or the hairdresser. The recruitment of the unexposed subjects is carried out during the compulsory medical examination within the occupational health centers and each eligible woman is proposed to participate in the study.

Ethics approval and consent to participate

This study was approved by the French Ethics Committee (Comité de Protection des Personnes Nord Ouest III, reference number: 2022-88). The processing of the study data has been the object of a declaration to the French Data Protection Authority on September 2021 (Commission Nationale de l'Informatique et des Libertés, registration number 2223653 v 0, 21 September 2021). Participation is voluntary and each participant is given an information letter to review before volunteering. Each subject provids written and informed consent to participate in the study.

Data collection

Data are collected at both individual and salon levels and at different time points - during the

inclusion visit within the occupational health center and during a 5-day work week in the hairdressing salon. The data collected at individual level concerns exposure assessment (paper questionnaires, urinary biomarkers of exposure to endocrine-disruptors, daily work sheets) and health outcomes (blood measurement of thyroid parameters). The data collected at the hairdressing salon level concerns the assessment of exposure throughout the salon visit (salon characteristics, inventory of chemical products).

During the inclusion visit, a questionnaire is administered face to face by the OHP to collect data about family history of thyroid disorders, personal use of current medicines, reproductive history, dermal disorders, hairdresser work characteristics and schedules, and the date of the last personal hair dyeing. Then, a self-administered questionnaire to collect information on sociodemographic and residence data, occupational history, alcohol, tobacco and food consumption, use of personal care and household products is given to the participant to be completed at home.

One blood sample is collected at the inclusion visit to measure health outcomes consisting in the thyroid hormone-related measures, including free and total T3 and T4, TSH, thyroglobulin (Tg), and thyroperoxidase antibodies (TPOAb) and thyroglobulin antibodies (TgAb). Because of the circadian rythms reported in the thyroid hormones [41], samples are collected for all participants in the morning during the same time slot (8 a.m.-12 a.m.). Serum TSH, free T3 and free T4 are measured by Access Immunoassay Systems with a DXI Beckman Coulter INC. analyzer (Ref. B63284 Access TSH 3rd IS ; Ref. A13422 Access Free T3 ; Ref. 33880 Access Free T4) [42]. Serum Tg and antibodies to TPO and Tg are measured by chemiluminescence electromagnetic immunoassays (ECLIA) with a Cobas e Roche Diagnostics immunoassay analyzer (Ref. 08906564190 Elecsys Tg II ; Ref. 07026935190 Elecsys Anti-TPO ; Ref. 09005021190 Elecsys Anti-Tg) [43]. Finally, total T3 and total T4 in serum are quantified by a chemiluminescent microparticle immunoassay (CMIA) on the Alinity i analyzer, a recently developed automated immunoassay analyzer [44].

Lastly, the protocol of 5-day work week follow-up is implemented to collect urines samples throughout the week, and to collect self reported data on the daily job tasks by means of daily work sheets to fill out at the workplace.

The exposure assessment is based on a multidisciplinary strategy including urinary biometrology, self-reported work organization and daily job tasks, and a visit of the hair salon including an inventory of the chemical products.

i. Individual exposure level
    a. Urinary measures

The urinary biometrology relates to the considered thyroid-disrupting chemicals of hair products,

namely resorcinol, parabens, and ultraviolet filters ethylhexyl methoxycinnamate and benzophenone. Considering the issues of toxicokinetics, the different sources and routes of exposure, as well as the individual variability of endocrine disruptors measures [45], the urinary samples are collected at different time points. Thus, the sampling framework take place at the beginning of the first day (day 1 preshift), at the beginning and at the end of day 3 and day 4 (pre- and post-shifts), and at the beginning of the last day of the work week (day 5 preshift) (Table 1).

Urinary measurements of resorcinol are assessed by a novel high performance liquid chromatography-high resolution mass spectrometry (HPLC-HRMS) method developed within our research team [46]. The lower limit of quantification is 0.3 µg/L resorcinol and the method was fully validated regarding accuracy, precison, carryover, linearity, recovery, and stability (raw samples and post-processing stabilities). The development and validation of urinary measurements of parabens (methyl, ethyl and propylparabene), ethylhexyl methoxycinnamate's metabolites (4'-methoxyacetophenone, acide 4-methoxycynnamic acid), and benzophenone-3 are currently in process.

b. Work organization and job tasks

Information on work organization and on daily job tasks are collected by questionnaires, at the inclusion visit, then during a 5-day working week by means of self-reported daily work sheets. Data on work organization relate to the seniority in hairdressing activity, other concomitant activities, type of employment contract (permanent, fixed-term, temporary), week schedules (number of working days, working hours and consecutive working days), date of the last working day. Information on daily job tasks relates to the activity during the 5-day follow-up week and consisted in the number of customers of the hairdresser and of the salon per day, the type and number of hairdressing tasks (shampoo, haircutting, dyeing, bleaching, perming, straightening, styling, modeling), the type and number of cleaning tasks at the workplace (tools, salon, furniture). For each hairdressing and cleaning task, additional questions are asked on the wearing of the personal protective equipment (gloves, facial mask), name of products used, the wearing of wrist/hand/fingers jewels, and smoking during the workday.

ii. Salon characteristics and inventory

A hair salon visit is carried out by 1 – 2 members of the study team in every salon where at least one haidresser was included in the study. The visit starts with an interview with the salon manager, followed by a brief observation of the salon and a full inventory of hair and chemical products within the salon. Information on work organization (staff, schedules, customers, hairdressing tasks) and salon characteristics (opening year, total surface, number and usage of rooms, customer seats,

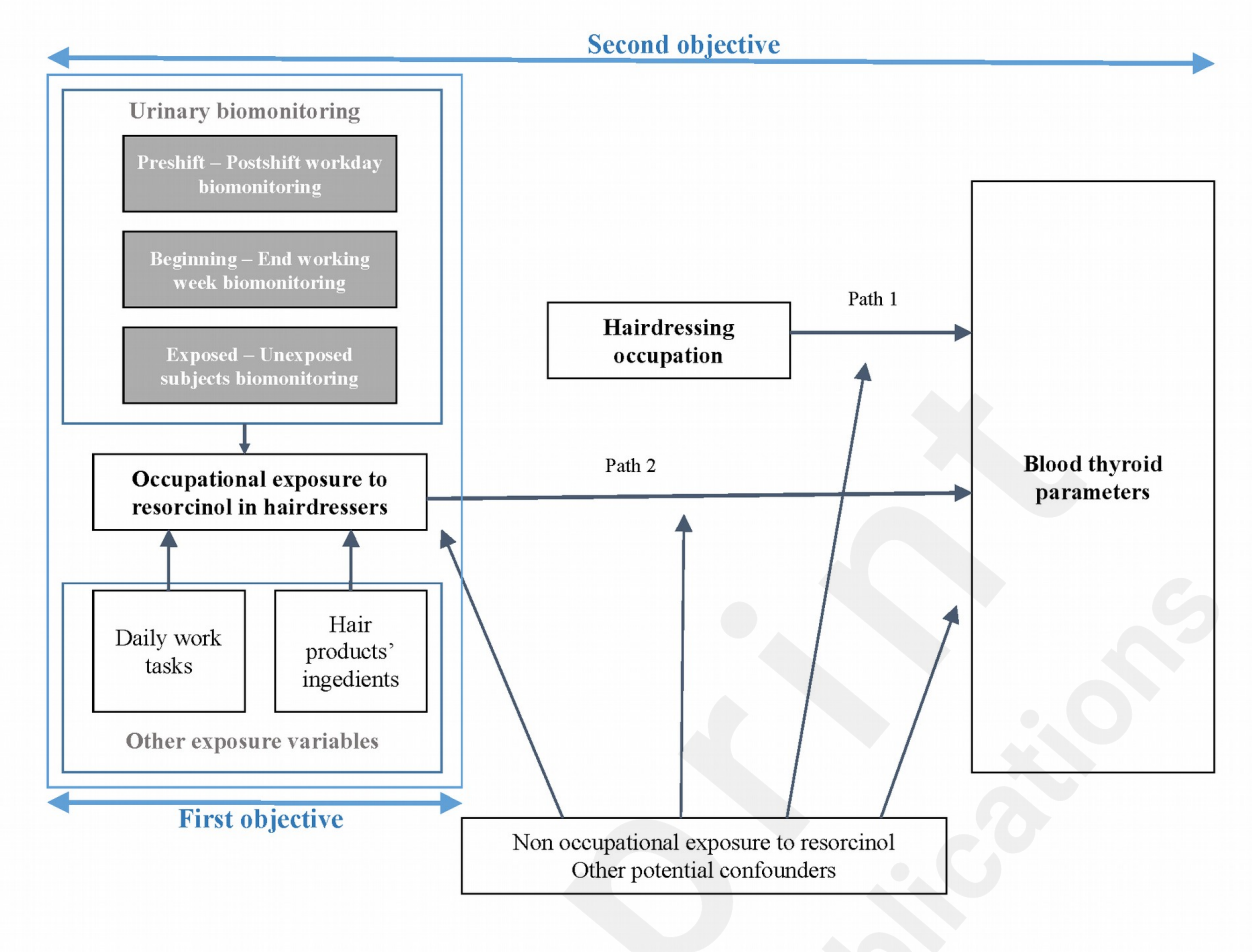
shampoo bins, ventilation systems, personal protective equipment, cleaning tasks of tools/salon/furniture, care and hair products, cleaning products) are collected. A full inventory of products in the salon is therefore undertaken by means of dedicated numeric tools - bar code scanner devices, mobile phones and a specific database held within our research team. The labels of all hair care and cleaning products are first scanned, then photos of the name and composition are taken for each product. This data is sent to the database to feed four sub-databases. The first sub-database contains all the products inventoried in the study, the second one all the products per salon, the third contains all the compounds per product, as mentioned in the label, and the fourth one a short toxicological information per compound according to the CAS number where it existed: IARC and CLP classifications for CMR [47, 48], CLP classification for irritants and sensitizers [48], belonging to three reference sources for endocrine disruptors. Namely, along with ED lists presented above [5], two other sources are consulted: the DEDuCT version 2 (Database of Endocrine Disrupting Chemicals and their Toxicity profiles) which compiles information on potential EDCs with supporting evidence from research articles published until January 2020 [49], and a list of substances of interest because of their potential endocrine action published by ANSES in 2021 [50].

Sample size and statistical methods

The sample size was determined on the basis of the mean serum levels of TSH (2.1 µUI/mL ± 1.0) and free T4 (10.9 ng/L ± 1.2) in healthy French women aged 35-60 years who were included in the Su.Vi.Max study [51]. So far, no data is available to estimate the differences in serum levels between hairdressers and the unexposed group, but we expect fairly small differences for free T4 (3-4%), and larger ones for TSH levels (13-15%).

For a ratio of 2 hairdressers for 1 unexposed subject, the population needed to detect these differences between hairdressers and unexposed consists in 300 hairdressers and 150 unexposed, and corresponds to an 80% power at a 5% significance level. Descriptive statistics will be used to describe all population and separately haidressers and unexposed subjects. To meet the study objectives, different statistical methods will be carried out according to the conceptual framework presented in Figure 3.

Figure 3. Conceptual framework following the study objectives



For assessing the occupational exposure to resorcinol among hairdressers, urinary concentrations of EDCs at the beginning of the working day will be compared to those at the end of the day, as well as for the concentrations measured at the beginning and the end of the working week, taking into account intra- and inter-individual variability (related to the repetition of samples over 2 days). Mean concentrations will be calculated with all samples collected during the week for exposed and unexposed population (up to 6 for exposed and 2 for non-occupationnally exposed) and compared between the two populations. An in-depth exposure assessment incorporating data from the hairdressing product inventory and daily work sheets will be carried out depending on the quality of the data. Non-occupational exposures to resorcinol described in litterature will be considered by using data relating to personal use of cosmetics, diet, alcohol consumption and smoking.

For analyzing thyroid disrupting effects in hairdressers, the relationship with thyroid parameters will be explored using two approaches. Firstly, by analyzing the association between hairdressing profession and thyroid hormone concentration using linear regression models (Path 1), taking into account potential confounding factors collected by questionnaire and linked to participant's residence, use of personal cosmetic products, consumption of food and drink, smoking, cleaning products use and occupational history. The different indicators of thyroid function will be treated

independently of each other or simultaneously using multivariate statistical models. Secondly, by analyzing the relationship between thyroid parameters and urinary impregnation with resorcinol and other EDCs for all urine samples combined, adjusted for confounders (Path 2), allowing to assess an "average" exposure but also the concentrations measured in the end-of-shift (post-shift) samples, in the hypothesis that the latter reflect a "maximum" exposure.

If possible, other individual exposure indexes will be determined on the basis of the daily work tasks crossed with the database of salon products and endocrine-disrupting substances. These indexes could be used to evaluate occupational exposures and to explore associations with thyroid parameters.

All analyses will be performed using Stata software (StataCorp LP, College Station, Texas).

Implementation schedule

Timeline of different steps

From 2022 the following main steps were carried out: regulatory approvals, the development of urinary measurement of considered thyroid disruptors, the implementation of the partnership with 15 occupational health centers, the achievement of data collection tools, the drafting of the operating mode for health professionals to follow. The inclusion of study population within the occupational health centers started in early 2023 and runs to end 2026.

Discussion

The present study is the first assessment of the occupational exposure to resorcinol in France, a thyroid disruptor relevant for human health. To the best of our knowledge, there is no published epidemiological studie that analyzed the relationship between occupational exposure to resorcinol and thyroid hormones. Considering the wide distribution of resorcinol in the environment and the significant increase of global resorcinol market over the forecast decade, we propose a timely biomonitoring and epidemiological study to provide new human data on exposure to resorcinol and other thyroid-disrupting chemicals in hair products.

The choice to recruit female subjects aged 18 - 45 having at least one year’s seniority in hairdressing and working in traditional hairdressing salons relies on the utility to built relatively homogene and stable groups concerning both exposure and thyroid parameters. Actually, the majority of hairdressers are women in childbearing age. Further, women older than 45 years exhibit more prevalent thyroid disorders. Finally, we can expect that occupational and non ocupational exposures to cosmetic products differ between the young apprentices and confirmed hairdressers. Data obtained from the 2007 to 2008 National Health and Nutrition Examination Survey (NHANES) showed that the relationships between thyroid hormones and endocrine disruptors were different between women and

men [52] and between adults and adolescents [53]. Moreover, the prevalence and pattern of thyroid disorders differ between the two genders [54], as well as the occupational exposure between traditional hairdressing salons and nail or beauty salons [24]. Lastly, even the construction of study population took into account the issues of statistical power and of feasibility, the results of the study can serve the whole occupational group including male hairdressers.

A strength of this study is its multilevel and multidisciplinary approach including epidemiology, occupational health, biometrology and endocrinology to set up the study methodology. Further, the collection of exposure data at hairdresser level and at salon level allows an extensive exposure assessment to thyroid-disrupting chemicals combining biometrology, industrial hygiene and inventory data exploitation. Specific partnership with occupational health centers was implemented for the recruitment process and data collection, and it is likely that their participation could improve the hairdressers' participation and the quality of data.

The study has a number of weaknesses. First, our study relies on salon managers' and hairdressers' willingness to participate in, so that the recruitment is very work-intensive and it may be difficult to achieve the expected number of subjects. To date, the participation rate ranges between 10-20% for hair salons and between 40-50% for hairdressers belonging to voluntary hair salons. The most common barriers reported by hair salon managers to the participation in the study are the over workload, problems with the labour force, the lack of interest for this research, concerns about the use of results (ie, hairdressers' health, job suitability, stock of hairdressing products), study procedures judged as too complicated, hairdressers' refusal. Further, non-inclusion criteria related to recent reproductive events or thyroid disorders are quite frequently reported by hairdressers.

To improve the participation rate, different tools for a better communication with each stakeholder were implemented, such as flyer/poster to inform haidressers/controls about the study, a newsletter to share information with occupational health centers, and informal discussion with salons managers within the health centers.

A second drawback, related to the first, is that this population might not be considered representative of the total French hairdressers. Also, the occupational health centers are volunteer to participate and we should consider this biais for the issue of representativity as well. Nevertheless, we can point outt a wide participation of health centers scattered throughout the French regions.

As long as the results are considered in the extents of these limits, this study will provide insights on the occupational exposure to hair products and thyroid-disrupting effects, but remains exploratory.

In conclusion, the present research based on a multidisciplinary approach including biometrology and epidemiology will provide the first insight on occupational exposure to resorcinol in France and

will advance our understanding on its thyroid disrupting effects. As hairdressers represent a susceptible population of childbearing age, we propose a timely research in partnership with occupational health centers based on an extensive exposure assessment to thyroid disruptors contained in hair products used in hairdressing salons. Together with the inventory of hairdressing and other chemical products, these results may strengthen the tools for the chemical risk diagnosis and prevention in hair salons.

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Authors' contributions: AR led the manuscript writting and contributes to the implementation of the study within Occupational Health Centers; AE supervised the development and the validation of the urinary assessment of endocrine-disrupting products and substantially contributed to the conception and the design of the study; BA, and MIN substantially contribute to protocol conception and implementation, to data collection, data management and validation; FD was in charge of developing assays and validation of the urinary measurement of endocrine disruptors chemicals; RP is the project coordinator and critically revised the final draft.

Conflicts of interest: None declared

Abbreviations

ANSES: French Agency for Food, Environmental & Occupational Health & Safety (Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail)

CLP: Classification, Labelling, Packaging

CMR: Cancerogenicity, Mutagenicity, Reprotoxicity

DeDuCT: Database of Endocrine Disrupting Chemicals and their Toxicity Profiles

ED: Endocrine Disruptor

EDCs: Endocrine-Disrupting Chemicals

EU: European Union

HPLC-HRMS: High Performance Liquid Cromathography-High Resolution Mass Spectrometry

IARC: International Agency for Research on Cancer

NHANES: National Health and Nutrition Examination Survey

OHP: Occupational Health Professionals

REACH: Registration, Evaluation, Autorisation, and Restriction of Chemicals

SCCS: Scientific Committee on Consumer Safety

SVHC: Substance of Very High Concern

T3: Triiodothytronin

T4: Thyroxin

Tg: Thyroglobulin

TgAb: Thyroglobulin Antibodies

TH: Thyroid Hormones

TPO: Thyroperoxidase

TPOAb: Thyroperoxidase Antibodies

TSH: Thyroid-Stimulating Hormone

TRH: Thyrotropin-Releasing Hormone

UV: ultraviolet radiation

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