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Estrogenicity of Major Organic Chemicals in Cigarette Sidestream Smoke Particulate Matter

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Abstract: We previously found that cigarette sidestream smoke particulate matter (CSSP) could activate estrogen receptor ERα to generate estrogen-like tumor-promoting effects. This study sought to identify the compounds responsible for CSSP estrogenicity. We first identified the component compounds using a combination of GC-MS and mass spectral matching. Based on computational estrogenicity prediction, nine potential estrogenic compounds were selected for second GC-MS identification and quantification. Their estrogenic activities at levels detected in the CSSP were verified using an estrogen-responsive reporter assay. Only catechol, a possible human carcinogen, showed significant estrogenic activity, but the activity was too low to justify CSSP estrogenicity. Even so, the mixture of these compounds reconstituted according to their contents in CSSP produced almost one third of the estrogenic activity of CSSP. These compounds acted synergistically to induce greater estrogenic effects at levels without apparent estrogenic activities. Nicotine accounted for approximately 16% of the total CSSP mass. The high abundance raises concerns about nicotine toxicity, including potentially working together with estrogenic chemicals to promote tumor growth. In summary, this study presents a tiered testing approach to identify estrogenic chemicals. Although no individual components are accountable for CSSP estrogenicity, the low-dose mixture effects of CSSP components warrant public health concerns.

Keywords: cigarette sidestream smoke; compound identification; estrogenicity prediction; estrogen receptor

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1. Introduction

Cigarette smoke is a significant source of indoor pollutants. When released indoors, cigarette smoke can linger in the air and settle on surfaces, exposing nonsmokers to the harmful chemicals in the smoke. Since 1964, 2.5 million nonsmokers have died due to exposure to secondhand smoke. Children, pregnant women, and individuals with pre-existing health conditions are particularly vulnerable to the harmful effects of secondhand smoke [1]. Secondhand smoke is composed of mainstream and sidestream cigarette smoke. Sidestream smoke, released directly from the burning tip of a cigarette, makes up about 85% of secondhand smoke [2]. In addition, cigarette sidestream smoke particulate matter (CSSP) has been reported to be four times more toxic than mainstream smoke particulate matter on a per gram basis [3].

CSSP possesses estrogen-like properties [4]. The estrogenic activity of CSSP has raised concerns because of the probability of mimicking or disrupting the actions of estrogen in a variety of physiological functions and pathological processes, including lung cancer development. Indeed, estrogen is a risk factor for lung cancer. In comparison with men, women have a higher susceptibility to tobacco carcinogens and a greater chance of developing lung cancer at a young age (<50 years, usually not yet reaching menopause) [5,6]. Women who have never smoked also have a higher risk of lung cancer, primarily lung adenocarcinoma, than nonsmoking men. Serum estrogen levels are inversely associated with survival in

patients with non-small cell lung cancer (NSCLC) [7,8]. NSCLC tumor tissues also contain higher concentrations of estrogen (~2.2-fold) than non-neoplastic tissues from the same patients. Tumor estrogen concentrations are positively associated with tumor sizes in nuclear estrogen receptor ERα-positive patients, but not ERα-negative patients [9]. Our previous study demonstrated that CSSP generated from a leading brand of cigarette in Taiwan did not only activate the transcriptional activity of ERα as 17β-estradiol (E2, the primary form of endogenous estrogen), but also enhanced E2-induced ERα activity in human lung adenocarcinoma cells. Exposure to either E2 or CSSP significantly increased the proliferation and migration of ERα-expressing lung adenocarcinoma cells [4].

Polycyclic aromatic hydrocarbons (PAHs) bear structural resemblance to steroid hormones, such as E2. A number of PAHs have been shown to be able to bind and activate ERα in human breast cancer cells and rat uterus [10,11]. We examined the contents of 22 PAHs in CSSP. In total, 17 PAHs were detected, constituting about 0.022% of the entire mass [12]. However, the reconstituted PAH mixture failed to activate ERα as CSSP [4]. Metals such as Al, As, Ba, Cd, Ni, and Pb can also induce estrogen-mimetic signaling via the activation of ERα, hence increasing the estrogen-like cancer risk [13]. Likewise, we confirmed that the estrogenic activity of CSSP is not attributable to metals found in CSSP [14]. This study aims to identify the component compounds contributing to the estrogenicity of CSSP. A tiered testing approach is taken in this study. We detect a synergistic mixture response among compounds without apparent estrogenic activities.

2. Materials and Methods

2.1. Preparation of CSSP Extracts

The CSSP was prepared from Long Life cigarettes, a leading brand in Taiwan, following a standard smoking procedure approved by the USA Federal Trade Commission and the International Standard Organization [15]. A 47 mm glass fiber filter with a pore size of 1 μm (Life Sciences, East Hills, NY, USA) was used to capture CSSP. After the measurement of the net dry weight of the CSSP absorbed on the filter, the CSSP was ultrasonically extracted from the filter using 20 mL methanol (Sigma-Aldrich, St. Louis, MO, USA) for 30 min. The extract was dried using the Savant Speed Vac SPD121P (Thermo Fisher Scientific, Waltham, MA, USA) at 1200 rpm and 40 °C. The vacuum-dried CSSP was then dissolved in ethanol (Sigma-Aldrich) to 180 mg/mL.

2.2. Compound Identification by Gas Chromatography–Mass Spectrometry (GC-MS)

After a ten-fold dilution with ethanol, two CSSP extract samples were analyzed using an Agilent 7890B GC system coupled with Agilent 5977A MSD (Agilent Technologies, Santa Clara, CA, USA). The GC was equipped with an Agilent HP-5MS column (60 m × 250 μm × 0.25 μm) using helium as the carrier gas, which flowed at a constant rate of 1.4 mL/min. The oven temperature was held at 80 °C for 2 min, and then elevated to 300 °C at a speed of 6 °C/min and maintained at 300 °C for 18 min. The inlet was maintained at 300 °C. The samples were injected at 1 μL with a split ratio of 50:1. The mass spectrometer was operated in the electron ionization mode at 230 °C with an ionizing voltage of 70 eV. Data were acquired in the scan mode with a mass range of 45–650 *m*/*z*. Component organic compounds were tentatively identified by searching the National Institute of Standards and Technology (NIST) Mass Spectral Database NIST11.

Component compounds with potential estrogenicities were verified and quantified by the GC-MS analysis described above, except that the samples were injected in a 5:1 split mode using a Gerstel autosampler (Gerstel GmbH, Mülheim, Germany). The ionic signals of standard compounds were profiled at multiple concentrations using the scan mode (45–650 *m*/*z*). The concentrations of analytes were measured against the corresponding calibration curves ($R2 > 0.995$) under the selected ion monitoring mode that was determined based on the ion profile of standards. Reference-standard compounds of catechol and hydroquinone were purchased from Nihon Shiyaku Reagent (Kobe, Japan), 4-ethylphenol from Dr. Ehrenstorfer (Augsburg, Germany), 2-phenylphenol, 2-methoxy-4-vinylphenol,

methoxyhydroquinone, and nicotine from Sigma-Aldrich, 2-pyrrolidinone from Alfa Aesar (Heysham, Lancashire, UK), and triacetin from Nippon Shiyaku (Kyoto, Japan).

2.3. Computational Estrogenic Potential Prediction

The estrogenic potentials of the tentative component compounds were estimated using the ToxCast ER model and the CERAPP model publicly available in the United States Environmental Protection Agency (US EPA) CompTox Chemicals Dashboard [16,17]. The dashboard also provides open access to the data of 48 ERα bioassays developed by the Toxicology in the 21st Century Program (Tox21), the Center for Computational Toxicology and Exposure (CCTE) Laboratories, and companies under contract to the US EPA such as ACEA Biosciences, Attagen (ATG), Novascreen (NVS), and Odyssey Thera (OT). The AC50 (half-maximal activation concentration) values derived from multi-concentration experiments are provided for active hit calls.

2.4. Estrogenic Activity Measurement by Estrogen-Responsive Reporter Assay

The estrogenicities of CSSP component compounds were examined by the transfection of an estrogen-responsive reporter, ERE-Luc, into an ERα-inducible cell line engineered from the human lung adenocarcinoma CL1-5 cell line in our laboratory [18]. The ERα-inducible cell line was routinely maintained in RPMI-1640 medium (Thermo Fisher Scientific) containing 10% fetal bovine serum (FBS, Thermo Fisher Scientific). To reduce the background noise in the estrogenic activity measurement, the cells were cultured in phenol red-free RPMI-1640 (Sigma-Aldrich) and charcoal/dextran-stripped FBS in experiments. The transfection was performed using Lipofectamine 2000 (Thermo Fisher Scientific) following the manufacturer's protocol. In addition to ERE-Luc, a β-galactosidase expression plasmid, pSV40-βgal, was co-transfected into cells as the transfection efficiency control. After 8 h transfection, the cells were rinsed and cultured in phenol red-free RPMI-1640 plus 3% charcoal/dextran-stripped FBS overnight before the induction of ERα expression by adding 1 μg/mL doxycycline (Sigma-Aldrich). Then, 24 h after doxycycline addition, the ERα-induced cells were further treated with 1 nM E2 (Sigma-Aldrich), 20 μg/mL CSSP, 1% dimethyl sulfoxide (vehicle control, Sigma-Aldrich), individual component compounds at concentrations found in 20 μg/mL CSSP, and the mixture of component compounds (OCM) for 24 h. Upon being activated by E2 or estrogenic compounds, ERα could bind to the estrogen-responsive element (ERE) and consequently induce the expression of the downstream luciferase reporter gene (Luc), which was manifested by its enzyme activity. Luciferase reporter activity was determined by normalization to the corresponding β-galactosidase activity, and the activities of both enzymes were measured using the Promega Luciferase and β-Galactosidase Assay Systems (Promega, Madison, WI, USA). The experiments were performed in three or four replicas as indicated in the figure caption. The reporter activity in relation to the vehicle control was calculated and expressed as mean ± SE. The significances of differences between the treatments ($p < 0.01$) were assessed by one-way ANOVA followed by Bonferroni's post hoc test (SPSS Statistics 19, IBM Corp., Armonk, NY, USA).

3. Results

3.1. Preliminary Mass Spectrum-Based Compound Identification

Prior to the identification of the unknown organic components of CSSP by GC-MS, two lengths of HP-5MS column (30 m vs. 60 m × 250 μm × 0.25 μm) were evaluated using GC-FID. The chromatogram of CSSP acquired from the 60 m column displayed superior resolution than that from the 30 m column. The 60 m HP-5MS column was used in the GC-MS analyses performed in this study. To increase the accuracy of unknown compound identification, two independent CSSP extracts were analyzed. The peaks that were detected in both extracts were identified by searching for matching mass spectra in the NIST Standard Reference Database. Thirty compounds exhibited a match percentage greater than 75% (Table 1), and each compound's highest match percentage score between the two samples

is provided. Additionally, Table 1 lists the retention time for each tentatively identified compound, indicating the amount of time it was eluted from the 60 m HP-5MS column following the sample injection.

Table 1. Preliminary chemical identification by GC-MS and mass spectral matching.

Retention(min) [1]	Compound	NISTMS Number [2]	Match(%) [3]
7.98	Phenol	133,909	91
9.65	o-Cresol	228,359	95
9.94	2-Pyrrolidinone	227,720	78
10.08	p-Cresol	395,159	97
10.54	3-Pyridinol	829	87
11.02	2-Methyl-3-pyridinol	33,210	83
12.25	4-Ethylphenol	341,131	93
12.92	Catechol	227,771	96
13.40	1,4:3,6-Dianhydro-*α*-*d*-glucopyranose	98,148	97
13.43	2,3-Dihydrobenzofuran	229,752	80
14.57	Hydroquinone	228,148	95
15.45	Indole	353,133	93
15.85	2-Methoxy-4-vinylphenol	135,956	83
16.26	Methylhydroquinone	229,907	93
16.35	Triacetin	229,309	83
	1,2-Diacetin	133,770	83
16.63	2,6-Dimethoxyphenol	231,854	92
16.72	Nicotine	232,303	97
17.54	3-Methylindole	228,764	93
18.39	Myosmine	109,884	96
19.58	Nicotyrine	109,886	93
20.66	2,3′-Bipyridine	229,245	97
20.74	4,4′-Bipyridine	228,653	94
23.18	2-Phenylphenol	113,331	81
24.00	Cotinine	334,060	97
24.75	9-Fluorenone	229,079	83
25.55	Phenanthrene	113,931	94
26.01	Phytyl acetate	375,014	83
27.43	Methyl hexadecanoate	333,716	98
28.62	β-Carboline	1,006,882	91

[1] The amount of time each tentatively identified compound remained in the 60 m HP-5MS capillary column equipped within GC-MS after the injection of a cigarette sidestream smoke particulate sample. [2] The number of reference mass spectra registered in the National Institute of Standards and Technology Standard Reference Database. [3] The match percentage between the query and reference spectra.

3.2. ER-Modulating Potential Prediction

To facilitate the identification of estrogenic component compounds, the ER-modulating potentials of the compounds listed in Table 1 were estimated by the ToxCast ER model and the CERAPP model. The ToxCast ER model incorporates results from 18 ToxCast ER assays to improve model prediction. This model scores estrogenicity by calculating the area under the concentration–response curve (AUC), which ranges from 0 to 1. AUC values <0.001 are considered to be null in ER activity and given a score of 0 in the ToxCast ER score system [16]. Four tentatively identified compounds have an ER agonist score >0.001. They are, from high to low score, triacetin, catechol, 2-phenylphenol, and 4-ethylphenol (Table 2).

Table 2. Forecasting the ER-modulating potentials of putative organic components in cigarette sidestream smoke particulate matter.

Compound [1]	CAS [2]	ER Modulation Prediction [3]			ERα Agonist Activity [3]
		ToxCast ER Model [4]	CERAPP [5]		
			Literature	Consensus	
Catechol	120-80-9	0.0155	–	–	Active
4-Ethylphenol	123-07-9	0.00352	–	Antagonist: very weak Binding: very weak	Active
Hydroquinone	123-31-9	0	–	–	Active
2-Phenylphenol	90-43-7	0.00543	–	Agonist: weakAntagonist: very weak Binding: weak	Active
2-Methoxy-4-vinylphenol	7786-61-0	–	–	Agonist: weak Antagonist: very weak Binding: weak	–
Methylhydroquinone	95-71-6	–	Binding: very weak	–	–
Nicotine	54-11-5	0	–	–	Active
Phenanthrene	85-01-8	0	–	–	Active
2-Pyrrolidinone	616-45-5	0	–	Binding: very weak	Active
Triacetin	102-76-1	0.0182	Binding: very weak	–	Active

[1] Candidate compounds that show positive results in predictive modeling or bioactivity screening are listed. [2] The registration number in the Chemical Abstracts Service (CAS). [3] Prediction results and activity data are acquired from the CompTox Chemicals Dashboard, a freely accessible online database created by the US EPA. [4] Agonist activity prediction by area under curve (AUC) scores. [5] The Collaborative Estrogen Receptor Activity Prediction Project (CERAPP) evaluates the ER-modulating potential of chemicals based on literature data and consensus modeling. Only "active" results are indicated.

The CERAPP consensus model is formed by the integration of 40 categorical models and 8 continuous models derived from different quantitative structure–activity relationships and structure-based docking predictions. The CERAPP program (Collaborative Estrogen Receptor Activity Prediction Project) also curates the ER-related experimental data in the literature. The CERAPP predictions are categorized into three groups: ER binders, ER agonists, and ER antagonists, and their respective potencies of binding, agonistic activity, and antagonistic activity are evaluated by comparing them to a library of 45 reference chemicals [17]. While methylhydroquinone and triacetin are known to have very low binding affinities to ER based on literature reports, CERAPP modeling predicts that 4-ethylphenol, 2-phenylphenol, 2-methoxy-4-vinylphenol, and 2-pyrrolidinone have the potential to bind, agonize, or antagonize ER. However, all of the predictive binding, agonistic, and antagonistic potencies are weak or very weak (Table 2).

The US EDSP21 (Endocrine Disruptor Screening Program in the 21st Century) and Tox21 programs have employed high-throughput biochemical- and cell-based assays to screen more than 10,000 chemicals for potential endocrine-disrupting or toxic bioactivities. Assay data are collected and curated in the EPA's CompTox Chemicals Dashboard [19,20]. Our search results indicate that except 2-methoxy-4-vinylphenol and methylhydroquinone, the compounds listed in Table 2 all tested positive for ERα agonist activity. The bioassays and AC50 data are summarized in Table 3. The ATG_ERE_CIS and ATG_ERa_TRANS assays seem to have higher sensitivities to obtain active hit calls. Six of the eight compounds listed in Table 3 show positive results in these two assays. The ATG_ERE_CIS assay measures the level of reporter mRNA that is stimulated by endogenous human ERα through the *cis*-acting response element ERE, while the ATG_ERa_TRANS assay quantifies reporter mRNA that is induced by the hybrid transcription factor GAL4-ERα through the GAL4-binding sequence. It appears that the ATG_ERE_CIS assay is used to assess the agonistic effect of a compound on the holoreceptor, while the ATG_ERa_TRANS assay evaluates the impact on the *trans*-activating potential of the ERα ligand-binding domain [21].

Table 3. Summary of the ERα agonist activities of putative estrogenic component compounds determined by EDSP21 and Tox21 assays.

Compound	Assay	AC50 (μM) [1]
Catechol	ATG_ERE_CIS	21.42
	ATG_ERa_TRANS	84.26
	CCTE_Deisenroth_AIME_384WELL_LUC_Active_up	24.14
	OT_ER_ERaERa_1440	60.43
	OT_ER_ERaERb_1440	53.83
	TOX21_ERa_BLA_Agonist_ratio	50.16
4-Ethylphenol	ATG_ERE_CIS	163.42
	ATG_ERa_TRANS	73.75
	OT_ER_ERaERb_0480	54.04
Hydroquinone	ATG_ERE_CIS	42.85
	ATG_ERa_TRANS	65.44
	CCTE_Deisenroth_AIME_96WELL_LUC_Active-up	53.61
2-Phenylphenol	ATG_ERE_CIS	11.96
	ATG_ERa_TRANS	30.24
	CCTE_Deisenroth_AIME_384WELL_LUC_Active_up	56.06
	CCTE_Deisenroth_AIME _96WELL_LUC_Active_up	80.54
	OT_ER_ERaERb_0480	82.65
	OT_ER_ERaERb_1440	36.93
Nicotine	ATG_ERE_CIS	47.79
	ATG_ERa_TRANS	61.85
Phenanthrene	OT_ER_ERaERb_0480	42.52
	ATG_hERa_XSP2	5.40
2-Pyrrolidinone	TOX21_ERa_LUC_VM7_Agonist	46.66
Triacetin	ATG_ERE_CIS	104.15
	ATG_ERa_TRANS	39.31

[1] Data of AC50, the half-maximal activation concentration, are acquired from the CompTox Chemicals Dashboard, a freely accessible online database created by the US EPA.

3.3. Identification of Estrogenic Components

Our previous study found that phenanthrene is the most abundant PAH present in the CSSP extract generated from the Taiwanese brand [12]. However, the mixture reconstituted according to the contents of PAHs in CSSP lacks estrogenic activity [4]. Therefore, we excluded phenanthrene from the second GC-MS identification and quantification (Table 4). As expected, CSSP has a high nicotine content (3694.64 μg/cigarette), constituting about 16% of the total mass. The second most abundant component identified in the GC-MS analysis is catechol, which is possibly carcinogenic to humans (IARC Group 2B) [22]. The amount of catechol in CSSP is 294.54 μg per cigarette, only about 8% of the nicotine mass. Hydroquinone, the third most abundant component (221.55 μg/cigarette) identified here, is an IARC Group 3 carcinogen that may cause cancer in animals, but has no acknowledged carcinogenicity in humans [23]. On the contrary, 2-phenylphnenol is not detectable in CSSP (Table 4).

An estrogen-responsive reporter assay was used to evaluate estrogenicity. CSSP at 20 μg/mL significantly induced reporter activity at 1 nM E2, although to a lesser extent (Figure 1A). Among the identified components, only catechol exhibited significant estrogenic activity at the concentration detected in CSSP, but its activity was much lower than that of CSSP (2.56 ± 0.15-fold vs. 21.01 ± 0.62-fold). The organic chemical mixture (OCM) reconstituted from the identified components according to their contents in CSSP (Table 4) produced remarkably higher estrogenic activity than the additive sum of their individual activities (6.60-fold vs. 3.88-fold). OCM accounted for almost one third of the estrogenic activity of CSSP (Figure 1B). Our findings suggest that the components of CSSP act together

to activate estrogen receptors. The synergistic interaction may cause adverse estrogenic effects on people exposed to CSSP.

Table 4. Amounts of putative estrogenic components in cigarette sidestream smoke particulate matter.

Compound	μg/Cigarette [1]	μg/20 μg [2]	REL/TLV [3]	IARC [4]
Catechol	294.54	0.254	20 mg/m^3	2B
4-Ethylphenol	28.19	0.024		
Hydroquinone	221.55	0.191	2 mg/m^3	3
2-Phenylphenol	nd [5]	nd		
2-Methoxy-4-vinylphenol	28.96	0.025		
Methoxyhydroquinone	135.17	0.117		
Nicotine	3694.64	3.189	0.5 mg/m^3	
2-Pyrrolidinone	128.22	0.111		
Triacetin	157.57	0.136		

[1] Mass of cigarette sidestream smoke particulate matter (CSSP): 23.17 mg/cigarette. [2] μg per 20 μg of CSSP. [3] REL: National Institute of Occupational Safety and Health's recommended exposure limit; TLV: American Conference of Governmental and Industrial Hygienists' threshold limit value. [4] IARC: International Agency for Research on Cancer's classification: Group 2B, possible human carcinogen; Group 3, evidence of carcinogenicity is inadequate in humans and limited in experimental animals. [5] nd: not detectable.

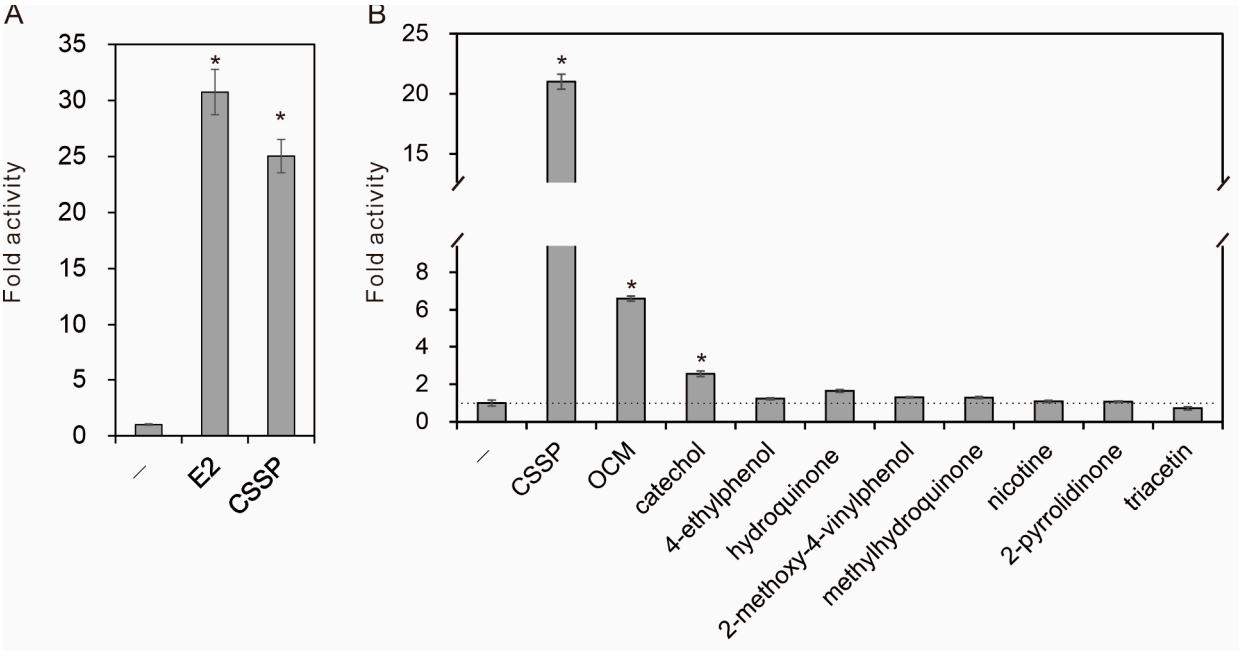

Figure 1. Estrogenic activity analysis. The estrogenic potencies of compounds and mixtures were assessed by transfection of an ERE-Luc reporter into ERα-inducible human lung adenocarcinoma cells. (**A**) The transfected cells were treated with vehicle (-), 17β-estradiol (E2, 1 nM), and cigarette sidestream smoke particulate matter (CSSP, 20 μg/mL) (n = 3). (**B**) Individual components of CSSP were tested at concentrations found in CSSP alongside their reconstituted mixture (OCM) and CSSP (n = 4). After 24 h of treatment in the presence of ERα induction, reporter activities were measured to evaluate the levels of ERα activation. All activities were compared with the vehicle control, and statistical significance ($p < 0.01$) was indicated by an asterisk (*).

4. Discussion

In addition to our research on CSSP, several studies have shown the presence of estrogenicity in cigarette smoke particulate matter generated from American, Japanese, and reference cigarettes. However, none of the latter studies sought to identify the specific components responsible for the estrogenicity or examined their estrogenic activity at the levels found in cigarette smoke [24–27]. Although we have not found individual components accountable for CSSP estrogenicity, we show that a number of CSSP components act together

to activate estrogen receptors synergistically. Previously, we demonstrated that CSSP can enhance ERα activity even when the receptor is saturated by E2 [4]. These component compounds may serve more of a function than acting as an ER ligand. The synergistic agonism may involve coactivator recruitment or the steric modification of ER [28,29]. It is also likely that some components in CSSP can induce certain enzyme activities that transform a component compound from pro-estrogenic to estrogenic [30].

Toxicological studies are generally aimed at single chemicals. However, the single-chemical practices fail to account for the combined effects of mixtures, particularly when components are present at low levels [31]. Our findings highlight the limitations of the conventional effect additive approach and the threshold concept in assessing the endocrine-disrupting properties of cigarette smoke. Epidemiological studies in humans have also demonstrated that exposure to mixtures of endocrine-disrupting chemicals (EDCs) can have a synergistic impact on health outcomes, such as obesity, diabetes, and reproductive disorders [32]. The regulation of EDCs should take the mixture effect and the potential for greater harm into consideration.

Catechol is the sole component in CSSP that demonstrates significant estrogenic activity at the detected level, but the activity is too low to justify the overall estrogenicity of CSSP. Although triacetin has a slightly higher ToxCast ER score than catechol (0.0182 vs. 0.0155), it is only present in half the amount of catechol in CSSP (157.57 μg vs. 294.54 μg per cigarette). In addition, triacetin had an AC50 almost five times higher than catechol (104.15 μM vs. 21.42 μM) in the ATG_ERE_CIS assay, which assesses the competence of a compound to activate human ERα using an ERE-reporter similar to the ERE-Luc reporter assay conducted in this study. Given these results, it is not surprising that triacetin did not induce the ERE-Luc reporter when tested on its own.

Catechol is not only an estrogenic chemical, but also a possible human carcinogen (IARC group 2B) [22]. Hydroquinone is also known to be carcinogenic in experimental animals (IARC group 3) [23]. In fact, more than 60 carcinogens, including strong carcinogens such as PAHs, nitrosamines, and aromatic amines, have been detected in mainstream and sidestream cigarette smoke [33]. The modulation of ER signaling may modify the carcinogenic effects of tobacco carcinogens. At least, the induction of ERα signaling promotes the development of hormone-dependent cancers such as breast, endometrial, and ovarian cancers [34]. ERα activation is also critical for the proliferation, migration, cisplatin resistance, and tumor growth of human lung adenocarcinoma cells [4,35,36].

Despite not being a potent ER agonist, nicotine can efficiently act on nicotinic cholinergic receptors in the brain to trigger the release of dopamine and other neurotransmitters that produce pleasant feelings. Withdrawal symptoms such as craving can occur upon the decline in brain nicotine levels. Addiction to nicotine is a key cause for smoking-related health problems [37]. There is also evidence suggesting that nicotine can convert to carcinogenic nitrosamines endogenously in humans [38]. It has also been demonstrated that nicotine and estrogen additively increase the angiogenesis and tumor growth of human bronchioloalveolar carcinoma xenografts implanted in ovariectomized nude mice [39]. Accordingly, estrogenic chemicals may promote tumor development when working together with nicotine. Furthermore, nicotine can readily cross the placenta to affect fetal development, potentially causing congenital anomalies [40].

A recommended exposure level (REL) of 0.5 mg/m^3 for nicotine has been established based on the no-adverse-effect level (NOAEL) from a two-year inhalation rat study. To improve the protection of workers from potential harm, the Health Council of the Netherlands has reviewed the study and proposed a health-based occupational exposure limit of 0.1 mg/m^3. This new limit is determined by reducing the exposure duration from 20 h to 8 h per day and incorporating an uncertainty factor of 9 for interspecies variation [41]. Considering the Dutch guideline and the high nicotine content in CSSP (3694.64 μg/cigarette), it is possible for individuals to be exposed to harmful levels of nicotine if they smoke two cigarettes simultaneously or consecutively in a poorly ventilated room of 60 m^3 (4 m × 5 m × 3 m).

5. Conclusions

Indoor pollution caused by secondhand smoke is a significant issue in homes, workplaces, and public spaces such as restaurants and bars. Exposure to secondhand smoke can pose serious health risks to individuals. The estrogenic activity of secondhand smoke is one of the subjects of concern. In this study, we employed GC-MS analysis, ToxCast ER model, and CERAPP model to identify potential estrogenic component compounds in CSSP. The ER-modulating capabilities of these components were further identified by an estrogen-responsive reporter assay. Only catechol was found to exhibit significant estrogenic activity at the detected level, but its activity was too low to justify the estrogenic capacity of CSSP. The mixture reconstituted according to the contents of these components in CSSP produced much higher estrogenic activity than the additive sum of the activities displayed by the individual components. The mixture activity accounted for almost one third of the CSSP estrogenicity. The synergistic agonistic action of CSSP components on estrogen receptors, even at levels below the thresholds, underscores the potential endocrine-disrupting risks associated with secondhand smoke exposure. In addition to estrogenicity, CSSP components have other toxic effects, such as (pro-)carcinogenicity, tumor promotion, and teratogenicity. The interaction of various effects of components determines the outcome of secondhand smoke exposure. More research is needed to understand the complex interaction. Our findings highlight the limitations of traditional toxicological approaches that focus on single chemicals and neglect mixture effects. Low-dose mixture effects need to be taken into consideration when assessing the health impacts of secondhand smoke.

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