UNIVERSIDADE
BEIRA INTERIOR

Efeito disruptor endócrino do triclosano na função vascular humana

Ana Catarina dos Santos Marques

Dissertação para obtenção do Grau de Mestre em
Biotecnologia
(2º ciclo de estudos)

Orientadora: Professora Doutora Maria Elisa Cairrão Rodrigues Oliveira
Coorientadora: Mestre Melissa Rodrigues Mariana

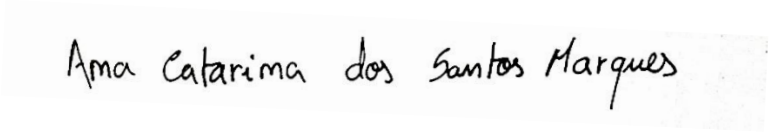
outubro de 2022

Declaração de Integridade

Eu, Ana Catarina dos Santos Marques, que abaixo assino, estudante com o número de inscrição M11092 de Biotecnologia da Faculdade de Ciências, declaro ter desenvolvido o presente trabalho e elaborado o presente texto em total consonância com o **Código de Integridades da Universidade da Beira Interior**.

Mais concretamente afirmo não ter incorrido em qualquer das variedades de Fraude Académica, e que aqui declaro conhecer, que em particular atendi à exigida referenciação de frases, extratos, imagens e outras formas de trabalho intelectual, e assumindo assim na íntegra as responsabilidades da autoria.

Universidade da Beira Interior, Covilhã 10 /10 /2022



(assinatura conforme Cartão de Cidadão ou preferencialmente
assinatura digital no documento original se naquele mesmo formato)

Dedicatória

Esta dissertação é dedicada a todas as pessoas que contribuíram para a minha decisão de realizar mestrado e que para além disso me apoiaram durante a sua realização.

Agradecimentos

A realização de uma dissertação representa não só muito trabalho individual, mas representa também uma etapa que não seria possível concluir sem o contributo de várias pessoas às quais eu gostaria de agradecer.

Em primeiro lugar, quero agradecer à Professora Doutora Maria Elisa Cairrão, por toda a disponibilidade, paciência, dedicação que sempre demostrou ao longo do meu percurso. E agradecer também por toda a motivação, a simpatia, o apoio e por todos os ensinamentos que me transmitiu.

Em segundo lugar, à Melissa, que para além de ser coorientadora ajudou-me a não perder a esperança, a vontade de trabalhar e por me ter aconselhado várias vezes.

Agradecer também à Fátima, à Francisca e à Margarida por todos os momentos passados a trabalhar como simplesmente a desanuviar do trabalho ao longo do ano.

Agradeço a todos os que viveram estes momentos comigo. Aos que estão presentes desde o início da caminhada, aos que foram aparecendo pelo caminho e aos que foram ficando pelo meio do caminho.

Aos meus pais por todo o apoio e sacrifícios que fizeram para que pudesse ser quem sou e por poder seguir aquilo que queria.

Obrigado

Resumo

Devido à pandemia o uso de desinfetantes tornou-se um ato importante do dia a dia. O triclosano (TCS) é um dos agentes antimicrobianos usados em desinfetantes, cosméticos, pastas de dentes e detergentes. Este composto é um disruptor endócrino querendo isto dizer que interfere com a função hormonal, tendo atividade estrogénica e androgénica. Estudos recentes demonstram que estes compostos podem ser responsáveis pelo desenvolvimento de várias patologias, nomeadamente a nível reprodutor, endócrino, neurológico e cardiovascular. Contudo, até à data os efeitos a nível vascular humano do TCS ainda não foram estabelecidos.

Tendo isto em conta, o objetivo deste trabalho consistiu no estudo do efeito do TCS no tónus arterial e na análise dos possíveis mecanismos envolvidos. Para atingir este objetivo foram delineados os seguintes objetivos específicos: 1) Análise do efeito direto do TCS na artéria umbilical humana sem endotélio; 2) Análise do efeito da exposição de TCS na contratilidade vascular; 3) Análise dos possíveis mecanismos envolvidos no efeito genómico do TCS na artéria umbilical humana, principalmente o envolvimento dos nucleótidos cíclicos e dos canais de cálcio.

Os resultados demonstraram que o TCS exerce um efeito contrátil arterial rápido (não genómico). O mecanismo de ação do TCS pode estar envolvido com a diminuição da síntese de óxido nítrico. Para além disso, neste mecanismo não envolve a ativação dos canais de cálcio do tipo L.

Em suma, o TCS parece prejudicar o sistema cardiovascular. Contudo, os efeitos a nível genómico permanecem por esclarecer. Assim, estudos adicionais são necessários para compreender melhor o mecanismo de ação do TCS a nível vascular.

Palavras-chave

Disruptor endócrino; Triclosano; Cardiovascular; Artéria umbilical Humana; Contração

Abstract

Due to the pandemic the use of disinfectants has become an important part of everyday life. Triclosan (TCS) is one of the antimicrobial agents used in disinfectants, cosmetics, toothpastes and detergents. This compound is an endocrine disruptor, meaning that it interferes with hormone function, having oestrogenic and androgenic activity. Recent studies show that these compounds may be responsible for the development of various pathologies, including reproductive, endocrine, neurological and cardiovascular. However, to date, the effects at the human vascular level of TCS have not been established.

With this in mind, the aim of this work was to study the effect of TCS on arterial tone and to analyse the possible mechanisms involved. To achieve this aim, the following specific objectives were outlined: 1) Analysis of the direct effect of TCS on human umbilical artery without endothelium; 2) Analysis of the effect of TCS exposure on vascular contractility; 3) Analysis of the possible mechanisms involved in the genomic effect of TCS on human umbilical artery, mainly the involvement of cyclic nucleotides and calcium channels.

The results demonstrated that TCS exerts a rapid (non-genomic) arterial contractile effect. The mechanism of action of TCS may be involved with the decrease of nitric oxide synthesis. Furthermore, this mechanism does not involve the activation of L-type calcium channels.

In short, TCS seems to impair the cardiovascular system. However, the effects at the genomic level remain unclear. Thus, additional studies are needed to better understand the mechanism of action of TCS at the vascular level.

Keywords

Endocrine disruptor; Triclosan; Cardiovascular; Human umbilical artery; Constriction

Índice

Índice de figuras

Índice de tabelas

Lista de Acrónimos

5-HT – serotonina

ADP – adenosina 5'-difosfato

AhR – recetor de hidrocarboneto de arilo

AT – angiotensina

ATP – adenosina 5'-trifosfato

BPA – bisfenol A

cAMP – monofosfato de adenosina cíclico

cGMP – monofosfato de guanosina cíclico

CHUCB – Centro Hospitalar Universitário da Cova da Beira

cNOS – óxido nítrico sintase dependente de Ca^{2+} e calmodulina

DAG – diacilglicerol

E2 – estradiol

ED – disruptor endócrino

EDHF – fator hiperpolarizante derivado do endotélio

EDRF – fator de relaxação dependente de endotélio

eNOS – óxido nítrico sintase endotelial

ERα – recetor de estrogénio alfa

ET – endotelina

FSH – hormona folículo-estimulante

HIS – histamina

HUA – artéria umbilical humana

HUC – cordão umbilical humano

iNOS – óxido nítrico sintase independente

IP3 – inositol trifosfato

KCl – cloreto de potássio

LDH – lipoproteína de alta densidade

LH – hormona luteinizante

LTCC – canais de cálcio tipo L

Nif – nifedipina

NO – óxido nítrico

NOS – óxido nítrico sintase

P450 – citocromo P450

PGI2 – prostaciclina I2

PPARγ – recetor gamma ativado por proliferador de peroxissoma

PSS – solução salina fisiológica

SNP – nitroprussiato de sódio

SULT – sulfotransferase

TCS – triclosano

UGT – UDP-glucuroniltransferase

VSMC – células musculares lisas vasculares

Capítulo 1

Introdução

1.1- Disruptores endócrinos

Um disruptor endócrino (ED) é um químico sintético ou natural que pode alterar vários aspetos da sinalização endócrina ao imitar ou inibir as ações das hormonas endógenas [1,2]. Pode alterar uma ou mais vias de sinalização hormonal, o que pode levar a efeitos negativos na reprodução, crescimento, sobrevivência, alterações comportamentais, indução de obesidade que podem ser transmitidos epigeneticamente para as próximas gerações [3]. Alguns dos EDs mais conhecidos incluem bifenis policlorados, dioxinas, alquifenois, ftalatos e bisfenol A [2].

A exposição a estes compostos pode ser por via oral através do consumo de comida ou água, via dérmica, via respiratória, via intravenosa ou transferência biológica através da placenta e leite materno [4].

O entendimento dos mecanismos de ação destes químicos tem crescido exponencialmente. Primeiramente pensava se que só exerciam ações diretamente para com os recetores nucleares de hormonas como os recetores de estrogénios, recetores de androgénios, recetores de progesterona, recetores da tiroide e recetores retinóicos, entre outros. Contudo, atualmente já se sabe que eles também podem alterar as vias não genómicas. Assim, os EDs podem atuar por uma grande variedade de mecanismos para alterar as vias de sinalização hormonal que tanto podem estimular como inibir essas vias (Figura 1), podendo: imitar total ou parcialmente as hormonas endógenas (estrogénios, androgénios, hormonas da tiroide) levando a uma ultra estimulação; atuar como antagonistas (anti-estrogénios e anti-androgénios) ligando-se aos recetores das hormonas endógenas; interferir ou bloquear as hormonas endógenas ou os recetores destas [4].

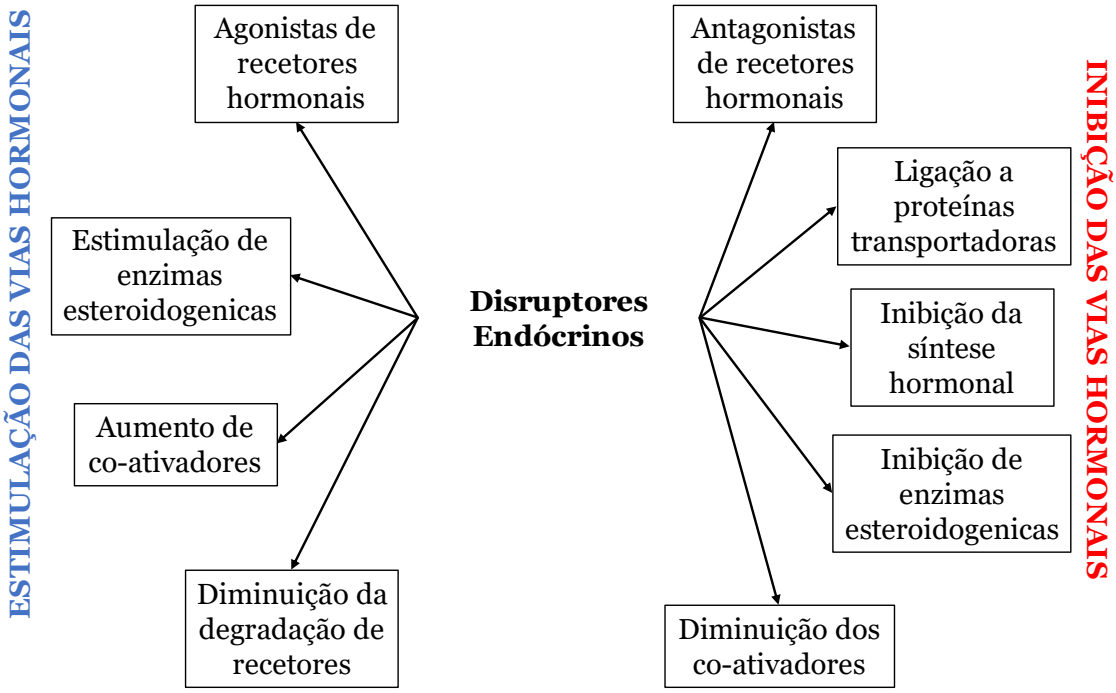


Figura 1: Mecanismos de disrupção endócrina

1.2- Triclosano

O Triclosano (TCS), 5-cloro-2-(2,4-diclorofenoxi) fenol, é um disruptor endócrino, que é comercialmente conhecido como Irgasan DP 300. Sendo um disruptor endócrino tem a capacidade de desregular a função endócrina [5], que devido à sua estrutura molecular pode ter efeitos similares a outros disruptores endócrinos, tais como bifenis policlorados, éteres difenilicos polibromados, bisfenol A (BPA) e dioxinas [6]. Especificamente, já foi reportado pela agência europeia de químicos que o TCS tem efeitos adversos a nível dos recetores de estrogénios e androgénios, da tiroide e alguns efeitos a nível cardiovascular [7].

O TCS tem sido amplamente usado em produtos de consumo diário desde 1968, nomeadamente como constituinte de produtos de higiene pessoal (sabões, pastas dentífricas, elixires bocais e produtos cosméticos), plásticos e têxteis (roupa antibacteriana) [8-11], tendo também sido implementado como revestimento de suturas [12]. As concentrações de TCS admitidas pela União Europeia são 0,1-0,3% (m/m) nos produtos de uso pessoal [13], estando de acordo com a concentração máxima admitida pelo Infarmed, 0,3% (m/m) [14]. No entanto, a *Food and Drug Administration* (FDA) considera o máximo admitido como 0% (m/m) desde 2019 [15].

1.2.1- Propriedades físico-químicas

O TCS é um pó branco cristalino, sem cheiro e sem sabor, com um peso molecular de 289,5 g/mol. É usado maioritariamente para aplicações orais [6,16]. Estruturalmente é uma molécula com grupos funcionais fenol éter (Figura 2), é lipofílico ($logk_{ow}$ = 4,8) sugerindo que este se acumula no tecido adiposo [17]. É um composto fenoxi-fenol clorado com um pka de 8,1. Não é hidrolisado facilmente, no entanto o pH, a presença de metais e matéria orgânica dissolvidos podem afetar a fotossensibilidade do triclosano [6].

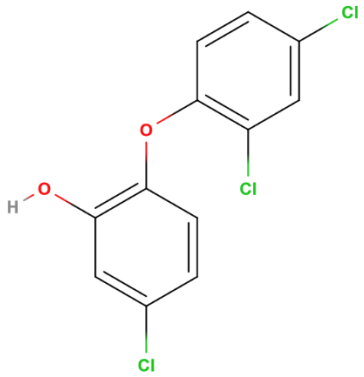


Figura 2: Fórmula estrutural do triclosano

É praticamente insolúvel em água, no entanto, é bastante solúvel na maioria dos solventes orgânicos não polares, como por exemplo acetona, etanol a 70% ou 95%, isopropanol, entre outros (Tabela 1 [18]).

Tabela 1: Tabela da solubilidade do triclosano em diversos solventes

Solvente	**Solubilidade a 25ºC (g Triclosano/ 100g solvente)**
Água destilada (20ºC)	0,001
Acetona	>100
Etanol 70% ou 95%	>100
Isopropanol	>100
Propileno glicol	>100
Tween 20	>100

O TCS é um agente antimicrobiano de longo espetro em que a sua atividade depende da sua concentração, isto é, a altas concentrações é bactericida e a baixas concentrações é bacteriostático. Tem um tempo de vida de aproximadamente 11 dias à superfície da água e é degradado em solo aeróbio com um tempo de meia vida de 18 dias [19].

1.2.2- Metabolismo

O TCS pode ser absorvido pela membrana mucosa da cavidade oral, por exposição dérmica ou através do trato gastrointestinal. É rapidamente absorvido pela pele devido às suas propriedades lipofílicas, sendo esta a principal via de absorção, pois a maior parte dos produtos que contêm TCS são para aplicação dérmica [20]. Lin et al. notaram que o TCS também pode ser absorvido oralmente, o que leva à deteção de metabolitos no plasma e provoca também a absorção via trato gastrointestinal [21]. Em humanos já foram detetadas concentrações de milimolar. Assim, e tendo em consideração as quantidades de TCS encontradas nos produtos de consumo humano, todas as vias de exposição são importantes para a exposição final [22]. Num estudo apresentado em 1992 [23], quando 1,6 mg de triclosano são aplicados diretamente na pele de ratos, este é rapidamente absorvido e o pico da concentração ocorre entre as 12 e 18 horas seguintes à exposição. Além desse estudo, Moss, Howes and Williams demonstraram que o triclosano penetra mais rapidamente pela pele dos ratos do que pela pele humana e que após 24 horas, só cerca de 12% da dose se encontrava na pele humana e cerca de 26% na pele de ratos [24].

No que concerne à distribuição desta substância, os estudos atualmente disponíveis dizem apenas respeito aos roedores. Kanetoshi e colaboradores foram os primeiros a demonstrar a metabolização desta substância a nível da bílis e do fígado. Os autores demonstraram que nas 24h seguintes à administração de TCS, os maiores níveis estavam presentes na bílis com 33,6 μg TCS/g de tecido, seguido do fígado que continha cerca de 3,0 μg TCS/g de tecido. As concentrações nestes dois tecidos mantiveram-se elevadas durante as 24h seguintes à administração aquando comparados com os restantes tecidos [23].

Noutro estudo realizado em ratos, este composto foi detetado nos 15 tecidos analisados 12h após aplicação cutânea, em que a vesicula biliar e a bexiga continham as concentrações mais elevadas enquanto os testículos, timo e cérebro apresentavam as concentrações mais baixas [25]. No mesmo sentido, Geens et al. extraíram tecido adiposo, tecido cerebral e fígado de 8 homens e 3 mulheres, através de autopsia, com idades compreendidas entre os 9 e 64 anos e administraram uma pequena dose de TCS. Estes

autores concluíram que o fígado e o tecido adiposo retinham melhor o TCS administrado que o tecido cerebral [26].

No referente ao metabolismo do TCS, este pode ser metabolizado por 3 vias: citocromo P450 (P450), UDP-glucuroniltransferase (UGT) e sulfotransferase (SULT) (Figura 3). Estas duas últimas fazem parte do metabolismo de xenobióticos de fase II [27,28].

O principal metabolito da hidroxilação do TCS é o triclosano monohidroxilado. Este, por sua vez, pode sofrer clivagem dando origem a 2,4-diclorofenol e 4-clorocatecol. Pode ainda ser formado um quarto metabolito a partir da hidroxilação do 2,4 diclorofenol formando assim o 3,5-diclorocatecol [29].

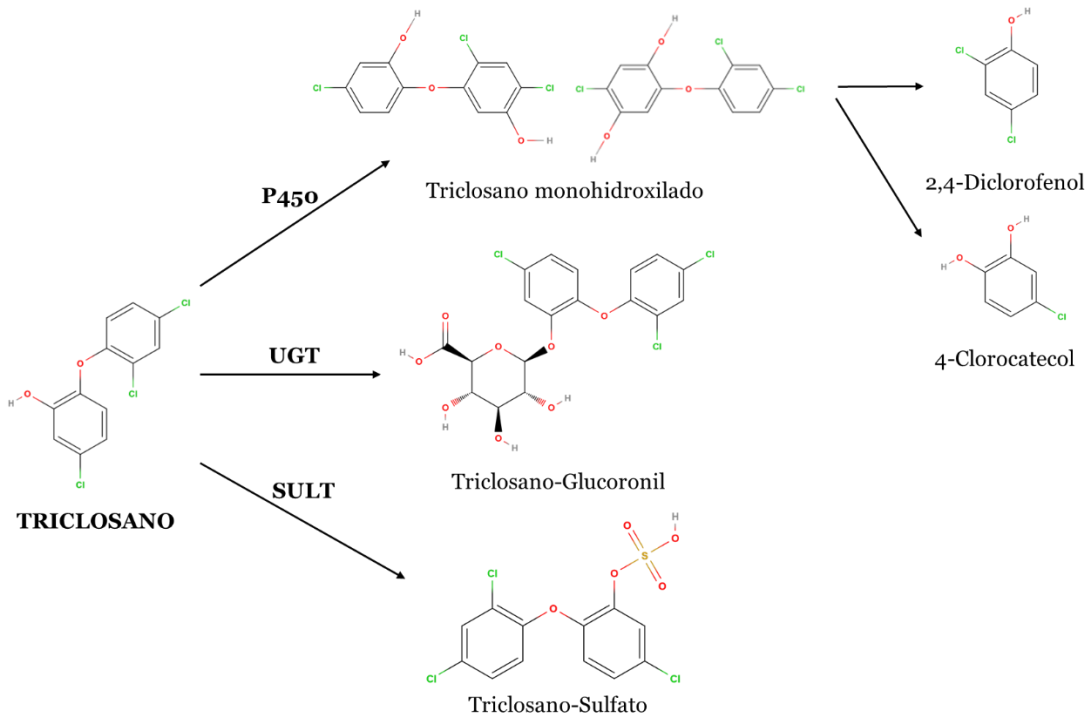


Figura 3: Metabolismo do triclosano e respetivos metabolitos

O facto de existirem duas vias de metabolismo de fase II não é influenciado pela via de administração, no entanto, a razão entre a transformação do TCS pelas duas vias já é influenciada pela espécie [17,30].

A glucuronidação e sulfatação do TCS provocam a adição de ácido glucurónico e sulfato, respetivamente, ao grupo hidroxilo, destruindo assim a natureza translocadora de protões e adicionando um grupo carregado/polar ao TCS [11].

O fígado é a maior fonte de metabolismo de TCS, no entanto este também ocorre em níveis mínimos na pele. A pele metaboliza primeiramente pela via SULTs e, após 24

horas da administração encontram-se ambos metabolitos de fase II. Apesar de ser metabolizado, a forma não metabolizada do TCS continua a ser a forma predominante [24].

No fígado, exposições de 1-5 μM de TCS por 30 min levaram a uma produção igual de TCS-sulfato e TCS-glucuronil, em exposições superiores a 20 μM predominou a glucuronidação enquanto doses inferiores a 1 μM levaram à sulfatação [31].

Num estudo realizado em 2014, 46 voluntários, (26 homens e 20 mulheres) com idades entre os 4 e 80 anos, foram testados e foi detetada tanto a presença de TCS-sulfato como TCS-glucuronil. O metabolito principal foi identificado como TCS-glucuronil [32].

No referente à eliminação do TCS, a principal via é a urina (57 a 87% da dose administrada) e a eliminação através das fezes é a via secundária (5 a 33% da dose administrada). Este composto é excretado principalmente nas formas metabolizadas: TCS-glucuronil e TCS-sulfato. Assim, durante os primeiros 4 dias após a exposição, entre 24 e 83% do TCS consumido é excretado, atingindo níveis de excreção basais após 8 dias [11,30,33].

O triclosano já foi detetado em várias matrizes biológicas, como urina, sangue, soro e leite materno (Tabela 2). Por esta razão têm sido realizados alguns estudos de forma a determinar se a presença do TCS pode afetar o feto, atravessando a barreira placentária, ou mesmo induzir efeitos adversos nas gerações futuras, como já foi observado para outros disruptores endócrinos [34-36].

Tabela 2: Concentrações de triclosano presentes nos fluidos biológicos

Fluído	Concentração (nM)	País	Referências
Soro	4,1-41,4	Espanha	[37]
Plasma	0,0035-1200	Austrália, Suécia	[20,38]
Urina	8,3-13090	EUA	[38,39]
	0,56±1,8 (não obeso)	India	[40]
	0,16±0,27 (obeso)		
	1,1-7,3	Espanha	[37]
	0,51±0,53	EUA	[41]
Leite materno	0,86-7,3	Espanha	[37]
	0,062-252	EUA, Austrália, Suécia	[38,42]

1.2.3- Efeitos do Triclosano em animais

O TCS pode afetar os animais a vários níveis, nomeadamente em peixes pela indução de apoptose em concentrações entre 0,16-0,25 mg/L ao aumentar os níveis de Bcl-2 diminuindo os níveis de Bax, como reportado por Liu et al. [43]. Também em peixes foi

reportada a capacidade do TCS para diminuir os níveis de testosterona, [44] o que não se verificou em ratos [45]. O efeito mais comummente observado em ratos foi a diminuição da concentração das hormonas sexuais: estradiol (E2), hormona luteinizante (LH) e hormona folículo-estimulante (FSH), e o aumento do número de abortos espontâneos maioritariamente relacionados com a alteração dos níveis de sulfotransferase de estrogénios [46-52].

Este composto pode também causar relaxamento das artérias aorta e mesentérica de rato previamente contraídas com fenileferina [53] causando diminuições no output cardíaco, no entanto, estes mostraram-se reversíveis após 24h [54]. Em relação ao peixe-zebra, o TCS reduz o ritmo cardíaco e a morfologia do coração, sendo também observadas alterações nos níveis de superóxido dismutase e lipoproteína de alta densidade (LDH) o que sugere a ocorrência de miocardite [55], dados que tinham sido previamente observados por Saley et al. [56]. Para além disso a exposição ao TCS aumenta a expressão de miR-181a-5p levando à diminuição dos vasos sanguíneos e das células sanguíneas em peixe-zebra [57].

Como a estrutura do TCS é similar à hormona da tiroide também é esperado que este interfira com a tiroide. Num estudo realizado em ratos, os níveis de tiroxina diminuíram após ingestão de 100, 300 e 1000 mg/kg/dia de TCS [58], resultados que foram verificados por outros autores, mas com concentrações inferiores [8]. Foi também observado que concentrações de 200 mg/kg provocavam pequenas alterações a nível da histologia da tiroide [59] e que a ingestão de TCS a 50 mg/kg provocou sintomas de hipotiroidismo também em ratos [60].

Em suma, o TCS parece estar associado à diminuição dos níveis de hormonas sexuais e aumento dos abortos espontâneos. Diminui o output cardíaco, leva à diminuição dos vasos sanguíneos e das células sanguíneas em peixe-zebra, também diminui os níveis de tiroxina, altera a histologia da tiroide e provoca sintomas de hipotiroidismo em ratos. Assim, o sistema reprodutor parece ser o mais afetado pela exposição ao TCS.

1.2.4- Efeitos do Triclosano na saúde humana

Em humanos foi possível verificar menores níveis de sulfotransferase de estrogénios quando detetadas altas concentrações de TCS, que correspondiam também à existência de abortos espontâneos [61]. Noutro estudo, a exposição ao TCS também levou à ocorrência de aborto espontâneo e à inibição de 11β-HSD2 da placenta [62]. Sabe-se também que o TCS influencia a mobilidade dos espermatozoides [63] e afeta a contagem de folículos antrais [64].

Estudos in vitro mostraram que as concentrações de TCS superiores a 50 μM afetaram a viabilidade de células endoteliais das veias do cordão umbilical, [65], e que em células estaminais embrionárias humanas uma exposição de 1 μM de TCS inibiu a diferenciação destas em cardiomiócitos [66].

Num estudo onde foi analisada a associação entre a concentração de 0,3% de triclosano em pastas de dentes e eventos cardiovasculares, foi observado que este composto não tem influência em pacientes com doenças cardiovasculares, a não ser uma diminuição do tempo decorrido até ao primeiro evento cardiovascular adverso grave [67]. Noutro estudo realizado pela mesma equipa concluiu-se que esta concentração de TCS nas pastas de dentes não tem uma influência significativa nos biomarcadores inflamatórios de doenças cardiovasculares [68].

Apesar de existirem alguns estudos que relacionam as concentrações urinárias de TCS com os seus efeitos a nível das hormonas da tiroide, estes são inconclusivos [69], uma vez que não observaram qualquer efeito [70]. Por outro lado, Berger et al. concluíram que existia uma relação inversa entre a concentração urinária de TCS e os níveis séricos de tiroxina total [71].

Em suma, o TCS parece afetar a mobilidade dos espermatoizoide e os folículos antrais, para além disso está associado a abortos espontâneos. A influencia cardiovascular só foi observada em estudos in vitro com a inibição da diferenciação celular. No entanto, os seus efeitos na tiroide não estão propriamente definidos porque apesar da concentração de TCS estar relacionada com diferenças nas concentrações das hormonas tiroideias os estudos são inconclusivos.

1.3- Cordão Umbilical Humano (HUC)

1.3.1- Características morfológicas

O cordão desenvolve-se a partir da vesícula vitelina e do alantóide. No início da gravidez, aproximadamente no dia 18, uma extensão da vesícula vitelina desenvolve-se numa haste de conexão, o alantóide transitório. Entre o dia 28 e 40 a cavidade amniótica expande-se, comprimindo o alantóide e a vesícula vitelina num cordão, criando assim o cordão umbilical [72]. O cordão vai aumentando o seu tamanho durante o primeiro mês é acompanhado por enrolamento, o seu comprimento médio é de 55 cm, podendo chegar aos 88 cm. É composto por duas artérias alantoides e duas veias alantoides, que se ligam à placenta durante a terceira semana, posteriormente durante o segundo mês de gravidez uma das veias alantoides sofre atrofia. Os vasos sanguíneos são necessários para a comunicação entre o feto em crescimento e a placenta. As duas artérias transportam

sangue pobre em oxigénio do feto para a placenta enquanto que, as veias devolvem sangue rico em nutrientes e oxigénio para o sistema circulatório do feto [73].

Durante o segundo mês de gravidez, a geleia de Wharton's é desenvolvida. É constituída por acido hialurónico, hidratos de carbono com grupos glicosil e manosil e previne a compressão e torção dos vasos constituintes do cordão umbilical [72].

Algumas das anomalias do cordão umbilical incluem cordões demasiado longos ou curtos, hiper ou hipoenrolamento, quistos, artéria umbilical singular, raramente um cordão ausente, tromboses nas artérias e veia, aneurisma da artéria, hematomas e tumores [72].

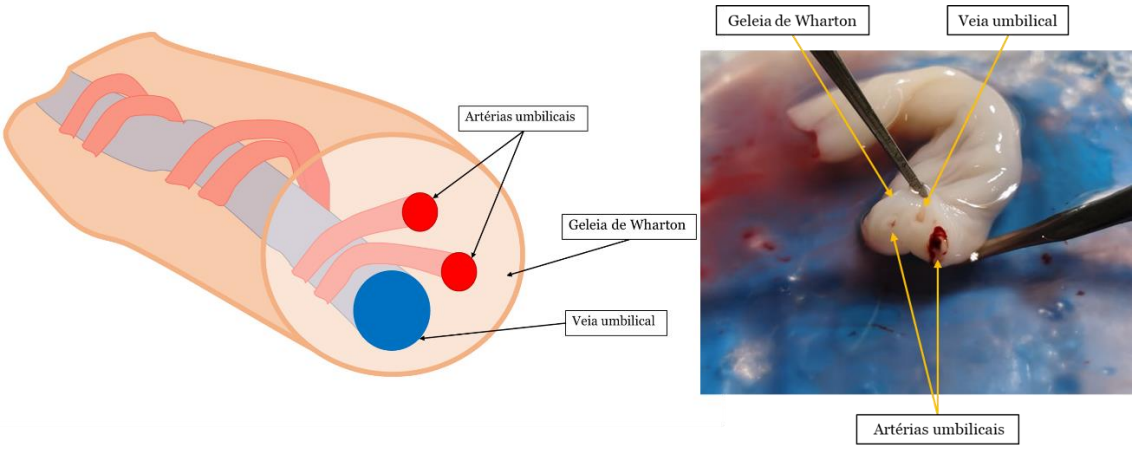


Figura 4: Esquema morfológico do cordão umbilical humano

1.3.2- Artéria umbilical humana (HUA)

A artéria umbilical humana (HUA) é uma artéria de calibre médio [35] que não contém inervação autónoma, devido a isso a HUA é regulada por mediadores locais como por exemplo prostaglandinas e serotonina (5-HT) ou alguns iões como potássio (K^+) e cálcio (Ca^{2+}) [74-76]. As artérias umbilicais são compostas por três camadas facilmente distinguíveis: a camada mais externa contém células musculares com um núcleo longo dispostas circularmente; a camada média é constituída por VSMCs distribuídas em duas camadas a mais externa é menos definida que a interna e apresenta uma disposição circular enquanto a mais interna tem uma aparência mais enrugada; e a camada interna contém células com núcleo obliquo o que obriga as células a apresentarem uma disposição em forma de hélice [35,77]. Todos os vasos sanguíneos, exceto os capilares, são compostos por uma estrutura de três camadas compostas pela túnica íntima, túnica média e túnica adventícia [78].

A túnica íntima, que é a camada mais interna, é composta por uma camada única de células endoteliais e uma camada fina de tecido conjuntivo. A túnica media é maioritariamente composta por células musculares lisas vasculares (VSMC) e produz a força mecânica e contrátil dos vasos sanguíneos, as células estão dispostas circularmente em forma de fuso rodeadas por uma matriz de colagénio e elastina. A camada mais externa, a túnica adventícia, é uma banda de tecido conjuntivo que fixa o vaso sanguíneo [79].

1.3.3- Células musculares lisas vasculares (VSMC)

As células musculares lisas vasculares podem ser isoladas a partir das artérias, nomeadamente da umbilical, sendo estas responsáveis pelos mecanismos de contração e relaxamento das artérias [79]. Desta forma são responsáveis pelo tónus vascular respondendo a vários estímulos hormonais e hemodinâmicos [35]. A contração e o relaxamento das VSMC dependem das substâncias vasoativas ou de fatores produzidos localmente pelas células endoteliais ou pelas células da geleia de Wharton [73]. O balanço entre os vasodilatadores e vasoconstritores determinam o tónus vascular, e a alteração deste balanço pode levar a um aumento de consequências pato-fisiológicas [79]. As VSMC contêm um grande número de filamentos de actina finos e um número menor de filamentos de miosina grossos que produzem contrações constantes e de baixa força relativamente ao músculo-esquelético.

1.3.4- Mecanismo de contração vascular

A contração das VSMCs acontece quando a concentração intracelular de Ca^{2+} aumenta e os vasoconstritores exercem as suas influências ao aumentar o Ca^{2+} e ou alterar a sensibilidade dos mecanismos de contratilidade ao Ca^{2+} intracelular [79]. Aumentos na concentração de Ca^{2+} intracelular ativam as cinases da cadeia de miosina, fosforilando as cadeias, o que faz com que a miosina interaja com a actina iniciando assim a contração do músculo liso [79]. O aumento da concentração de Ca^{2+} citosólico livre pode ser originado por um aumento rápido e transiente devido à libertação de Ca^{2+} do retículo sarcoplasmático ou por um influxo de Ca^{2+} por canais de Ca^{2+}, o que leva à ativação da cinase da cadeia de miosina e à contração devido à interação entre os filamentos de actina e miosina.

A serotonina induz contrações nas VSMC através do influxo de cálcio do meio extracelular a partir de canais de cálcio dependentes de recetores e da libertação de cálcio de reservas intracelulares, enquanto que as contrações devidas ao cloreto de potássio

(KCl) são maioritariamente devidas ao influxo de cálcio por canais de cálcio dependentes da voltagem ligados à despolarização da membrana [74].

Diferentes tipos de canais de Ca^{2+} estão envolvidos no controlo da contração das VSMC, como os canais tipo L e tipo T. Os canais tipo T estão maioritariamente envolvidos na proliferação celular, no entanto não existe muita informação que ligue estes canais à contractilidade das VSMC. Por outro lado, os canais tipo L são dominantes no que diz respeito à contractilidade das VSMC pois estão reportados como a via principal de entrada de Ca^{2+} [75,80].

As endotelinas (ET) são uma família de péptidos com 21 aminoácidos produzidas pelas células endoteliais e atuam no músculo liso vascular provocando vasoconstrição. Nos humanos foram identificados 3 recetores para as endotelinas ETα e ETβ2 responsáveis pela vasoconstrição e ETβ1 que leva a vasodilatação pela libertação de óxido nítrico. A vasoconstrição pela endotelina-1 está associada a um aumento na concentração intracelular de Ca2+. A ligação da endotelina-1 ao seu recetor ativa a fosfolipase C levando à produção de dois segundos mensageiros, o inositol trifosfato (IP3) e o diacilglicerol (DAG). O aumento de Ca^{2+} intracelular é promovido pela libertação das reservas de Ca^{2+} intracelular que são promovidas pelo IP3 e pelo influxo de Ca^{2+} através dos canais de Ca^{2+} dependentes da voltagem presentes na membrana. Apesar da endotelina-1 ser um potente vasoconstritor, esta também promove a libertação de outros fatores contráteis dependentes de endotélio [79].

A prostaglandina H2 e o tromboxano A2 têm a capacidade de se difundir nas VSMC estimulando os recetores endoperóxido/tromboxano aumentando a concentração de Ca^{2+} citosólico, provocando contração. No entanto a sua importância a nível fisiológico continua incerta [79].

A angiotensina II é outro vasoconstritor que atua pelo recetor AT1, o recetor AT2 está relacionado com a observação de problemas patológicos como enfarte do miocárdio e lesões na pele [79].

1.3.5- Mecanismo de relaxamento vascular

Os mecanismos de relaxamento das VSMC podem ser mediados pela ativação da fosfatase da cadeia leve de miosina, que tem o efeito oposto da cinase da mesma. Os nucleótidos cíclicos monofosfato de adenosina cíclico (cAMP) e monofosfato de guanosina cíclico (cGMP) atuam como segundos mensageiros, sendo as principais vias envolvidas na regulação da vasodilatação [75].

Várias substâncias são conhecidas por induzir relaxamento dependente de endotélio resultando na libertação de fatores de relaxação dependentes de endotélio (EDRF), que se pensa ser óxido nítrico (NO) [76]. O NO é sintetizado pela óxido nítrico sintase (NOS) que pode ser dependente de Ca^{2+} e calmodulina (cNOS), e independente (iNOS). A cNOS só se liga à calmodulina quando os níveis de Ca2+ intracelular são elevados, enquanto que a iNOS está fortemente ligada à calmodulina a níveis normais de Ca2+ intracelular (35-70 nM) [79]. NO tem um papel importante na regulação do tónus vascular, do tónus do músculo cardíaco, na inibição da agregação das plaquetas e também é um mediador de resposta imunológica. As ações biológicas do NO podem ser atribuídas à estimulação da guanilil ciclase solúvel e a subsequente produção de monofosfato de guanosina (GMP) cíclico e a ativação da cinase G, que se pensa ser o principal mecanismo responsável pelo relaxamento através do NO [81]. Estudos prévios comprovam que o relaxamento, em algumas células endoteliais, é acompanhado por uma despolarização da membrana. Essas células libertam um fator que provoca a hiperpolarização da membrana, o fator hiperpolarizante derivado do endotélio (EDHF) [82].

A vasodilatação dependente de endotélio provocado pelas plaquetas resulta da resposta de células endoteliais à adenina 5'-difosfato (ADP), adenina 5'-trifosfato (ATP) e serotonina.

A prostaciclina também chamada de prostaglandina I2 (PGI2) é produzida principalmente nas células endoteliais vasculares e na túnica íntima, com uma diminuição da produção no sentido da túnica adventícia [79]. A PGI2 exerce o seu efeito vasodilatador ao estimular o recetor IP de superfície da proteína G, que por sua vez ativa a adenilil ciclase aumentando os níveis intracelulares de cAMP. Com este aumento a proteína cinase A diminui a atividade da cinase da cadeia leve de miosina, inibido assim a contração [79].

Outro mecanismo de relaxamento é provocado pela pressão que a corrente sanguínea exerce nos vasos, isto é, em artérias de maior calibre quando o fluxo sanguíneo aumenta existe uma dilatação provocada por esse fluxo, atualmente o fenómeno é estabelecido como dependente de endotélio e pode contribuir para a libertação de fatores de relaxamento dependentes de endotélio (EDRF) [82].

Capítulo 2

Objetivos

Em animais os efeitos do TCS parecem estar associados a efeitos a nível reprodutor, tiroideo e cardiovascular. Estudos anteriores demonstraram que este composto é um disruptor endócrino, e que pode estar associado a problemas ou ao desenvolvimento de patologias cardiovasculares. No entanto, em humanos, o número de estudos realizados é muito escasso, sendo que os efeitos a nível cardiovascular são completamente desconhecidos. Assim o objetivo principal deste trabalho é verificar quais os efeitos que o triclosano poderá ter a nível cardiovascular, recorrendo às artérias do cordão umbilical sem patologia associada.

De forma a atingir o objetivo central foram definidos objetivos específicos:

1. Análise do efeito direto do TCS na HUA sem endotélio
2. Análise do efeito da exposição de TCS na contratilidade vascular
3. Análise dos possíveis mecanismos envolvidos no efeito genómico do TCS na HUA

Capítulo 3

Materiais e Metodologias

3.1- Recolha de cordões umbilicais humanos (HUC)

Todos os procedimentos realizados com os cordões umbilicais foram aprovados pelo Comité de ética do "Centro Hospitalar Universitário Cova da Beira E.P.E." (Covilhã, Portugal) e estão em conformidade com os princípios da Declaração de Helsínquia. As frações de cordão utilizadas para o estudo foram recolhidas no bloco de Obstetrícia e Ginecologia, obtidas de partos que ocorreram entre as 37 e as 42 semanas de gestação por via vaginal, com o consentimento informado das mães dadoras. As amostras de cordão foram colocadas numa solução salina fisiológica estéril (PSS) e mantidas por 4-24h, à temperatura de 4ºC. À solução de PSS foram adicionados antibióticos (penicilina (5 U/ml), estreptomicina (5 μg/ml) e anfotericina B (12.5 ng/ml)), e antiproteases (leupeptina 0.45 mg/l, benzamidina 26 mg/l, e inibidor de tripsina 10 mg/l), de forma a evitar possíveis contaminações e degradação tecidual, respetivamente.

3.2- Estudos de contractilidade arterial a partir da técnica de banho de órgãos

Começou-se por isolar as artérias do cordão umbilical de forma a efetuar os estudos de contratilidade arterial sem endotélio. Para iniciar o isolamento da HUA, o cordão umbilical foi colocado numa caixa de Petri, com PSS, e de seguida removeu-se cuidadosamente a geleia de Wharton. As artérias isoladas foram colocadas em 10 mL de PSS à temperatura de 0-4ºC. Passadas 3-12 horas o endotélio foi removido de forma mecânica pela fricção com uma linha de algodão introduzida através do lúmen arterial, e as artérias sem endotélio foram colocadas num falcon com 10 mL de DMEM, até à realização dos estudos.

As artérias isoladas anteriormente foram cortadas em pedaços retangulares com aproximadamente 3-5 mm (anéis). Seguidamente, os anéis das HUA foram colocados no banho de órgãos contendo solução de bicarbonato de Krebs a uma temperatura de 37°C, onde permaneceram em contacto com gás carbogénio (95% O2 e 5% CO2) de uma forma contínua. Os anéis foram suspensos entre dois ganchos paralelos de aço inoxidável. A medição da tensão isométrica foi feita utilizando transdutores de força (TRI201, Panlab SA, Espanha), um amplificador (ML118/D Quad Bridge, ADInstrumentos) e uma

interface PowerLab/4SP (ML750, ADInstruments) ligada a um sistema computorizado com o programa "PowerLab Chart5". Inicialmente e após a montagem dos anéis no banho de órgãos, estes foram submetidos a uma tensão basal entre 1,5-2,0g, e equilibrados durante 45 min (período de estabilização). Durante esse período, a solução fisiológica no banho foi trocada ao fim de cada 15 min, correspondendo aos tempos de lavagem.

Primeiramente os anéis da HUA foram contraídos com serotonina (5-HT, 1 µM) ou cloreto de potássio (KCl, 60 mM) e, após estabilização da resposta contráctil induzida pelo agente contráctil, o efeito vascular do triclosano (TCS: 0,01-10 µM) foi avaliado. As diferentes concentrações de TCS foram adicionadas de forma crescente, como demonstrado na figura 5, após a estabilização do efeito do agente contrátil. Foram realizados controlos com etanol (o veículo usado para dissolver o TCS) nas mesmas condições acima referidas.

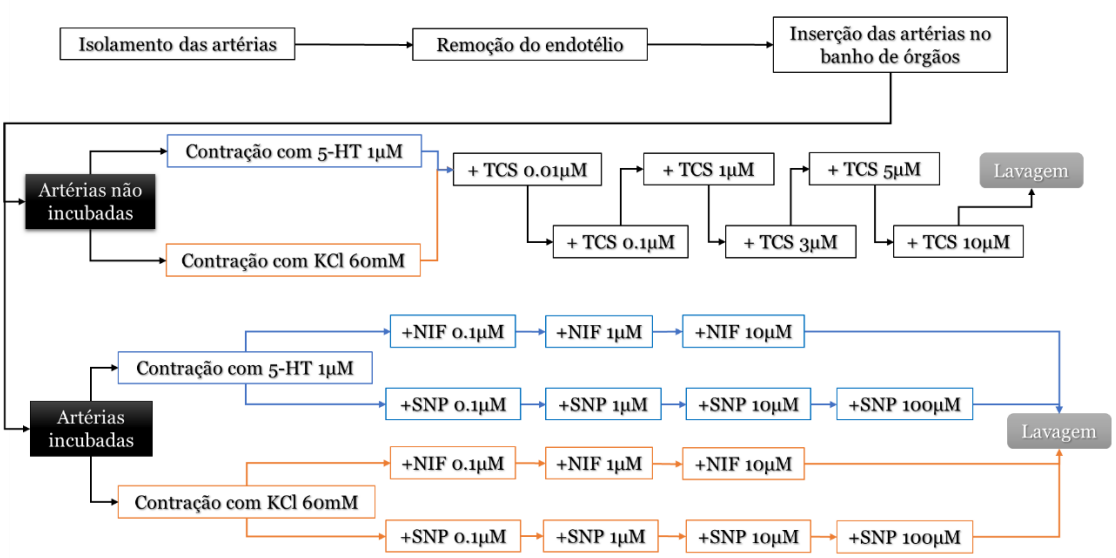


Figura 5: Esquema da realização do banho de órgãos

Para a realização do efeito genómico do TCS, após a remoção do endotélio, as artérias foram incubadas por 24 horas a concentrações de TCS 10 e 1000 µM. Os anéis de HUA foram contraídos utilizando dois agentes contrateis: serotonina (5-HT; 1µM) ou cloreto de potássio (KCl; 60mM). De seguida foram adicionadas de forma crescente concentrações de nifedipina (0,1; 1; 10 µM) e nitroprussiato de sódio (0,1; 1; 10; 100 µM) a artérias diferentes, como demonstrado na figura 5. Todo o procedimento anteriormente descrito foi realizado na ausência de luz, uma vez que a nifedipina e o nitroprussiato de sódio são agentes foto degradáveis.

3.3- Soluções

Tabela 3: Soluções utilizadas e respetiva composição

Solução	Composição
Solução salina fisiológica estéril (PSS) ou solução salina fisiológica estéril diluída	NaCl 110mM; $CaCl_2$ 0,16 mM; KCl 5mM; $MgCl_2$ 2mM; Hepes 10mM; $NaHCO_3$ 10mM; KH_2PO_4 0,5 mM; NaH_2PO_4 0,5 mM; Glicose 10 mM e EDTA 0,5 mM (pH=7,4)
Krebs Básico Concentrado	KCl 5,0 mM; EDTA 0,03 mM; $MgSO_4.7H_2O$ 1,2 mM; KH_2PO_4 1,2 mM e ácido L-ascorbico 0,6 mM
Krebs diluído	Krebs Básico Concentrado; $CaCl_2$ 0,5 mM; NaCl 119 mM; $NaHCO_3$ 25 mM e Glicose 11 mM (pH=7,4)
Krebs despolarizante (KCl 60 mM)	Krebs Básico Concentrado; $CaCl_2$ 0,5 mM; NaCl 64 mM; KCl 60,0 mM; $NaHCO_3$ 25mM e Glicose 11 mM (pH=7,4)

3.4- Materiais

Tabela 4: Materiais/equipamentos utilizados no decorrer desta investigação

Preparação de soluções	
Material	**Marca**
Balança digital	PLJ 510-3M, Kern
Medidor de pH	Metrohm
Banho de órgãos	
Câmara de Banho de órgãos	Letica
Transdutor de força	
Amplificador	ADInstruments
Interface PowerLab/4SP	
Software específico do banho de órgãos	
Material cirúrgico específico	F.S.T

3.5- Químicos

Para a elaboração desta investigação foram utilizados os seguintes compostos químicos: o triclosano (TCS), a serotonina (5-HT), nifedipina (Nif), nitropussiato de sódio (SNP) e

etanol (EtOH). Todos os compostos foram adquiridos através do Sigma-Aldrich Química (Sintra, Portugal). Inicialmente a serotonina e o nitroprussiato de sódio foram dissolvidos em água destilada, enquanto o TCS e a nifedipina, foram dissolvidos em etanol. Para as diversas metodologias realizadas foi necessário preparar diluições de TCS, NIF, SNP e EtOH. Nos estudos de contractilidade arterial as diluições foram realizadas em Krebs diluído. Todas as diluições necessárias foram sempre realizadas no próprio dia da experiência, de modo a não influenciar os resultados.

Capítulo 4

Resultados

4.1- Estudos de contratilidade arterial

Os seguintes estudos de contratilidade arterial de artérias umbilicais humanas (HUA) foram realizados recorrendo à técnica de Banho de Órgãos.

4.1.1- Efeitos vasculares diretos do TCS na contratilidade das HUA induzida pela serotonina (5-HT)

Anéis vasculares de artérias umbilicais humanas (HUA) foram contraídos com serotonina (5-HT, 1 μM), sendo atingidas contrações estáveis depois de 10-15 minutos (1947 ± 121,24 mg). O efeito vascular do triclosano (TCS 0,01; 0,1; 1; 3; 5 e 10 μM) sobre esta contração foi analisado.

O gráfico 1 resume os resultados obtidos nas experiências realizadas.

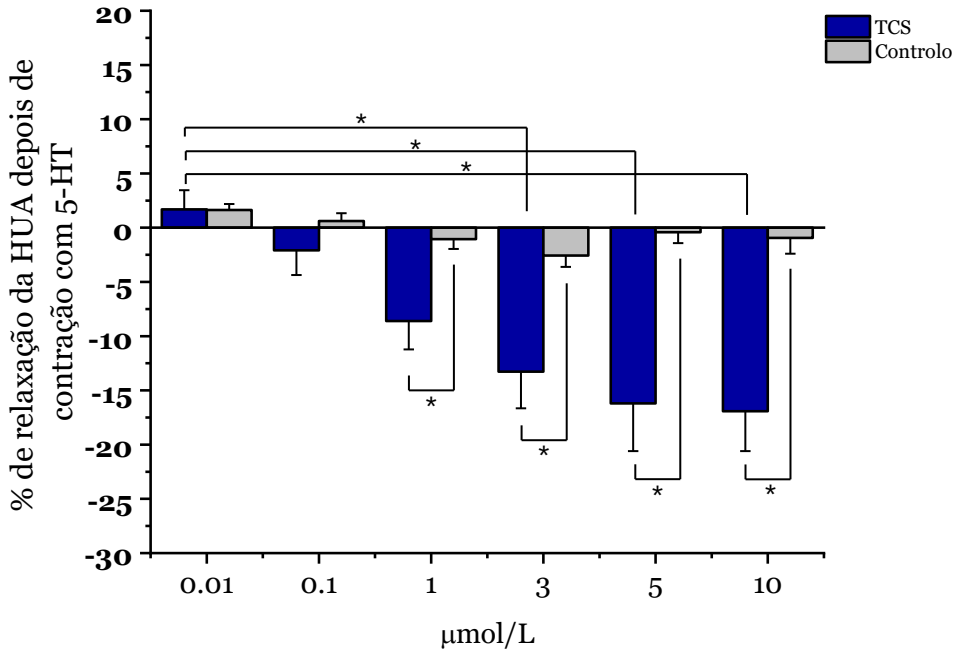


*Gráfico 1: Efeito do TCS (0,01; 0,1; 1; 3; 5 e 10 μM) sobre as contrações induzidas por serotonina (5-HT, 1 μM) em HUA. Os dados foram expressos em percentagem (%) de relaxação sobre a contração provocada pela 5-HT. As barras representam a média e as linhas verticais o erro padrão da média (SEM). A análise estatística foi realizada pelo t-student, onde * P<0,05 versus o controlo e pelo teste one-way ANOVA com teste post hoc Holm-Sidak, onde * P<0,05 versus concentrações de TCS.*

Como mostrado no gráfico 1, diferenças significativas foram encontradas entre o grupo controlo (EtOH) e o TCS 1, 3, 5 e 10 μM ($p<0,05$, teste t-student). O TCS 1, 3, 5 e 10 μM provocou um efeito vasoconstritor ($p<0,05$, one way ANOVA com teste post hoc Holm-Sidak), sendo a concentração de 10 μM aquela que provocou uma maior constrição vascular (16,921 ± 3,669 %).

4.1.2- Efeitos vasculares diretos do TCS na contratilidade das HUA induzida pelo cloreto de potássio (KCl)

Anéis vasculares de artérias umbilicais humanas (HUA) foram contraídos com cloreto de potássio (KCl, 60 mM), sendo atingidas contrações estáveis depois de 10-15 minutos (2000,52 ± 102,64 mg). O efeito vascular do triclosano (TCS 0,01; 0,1; 1; 3; 5 e 10 μM) sobre esta contração foi analisado.

O gráfico 2 resume os resultados obtidos nas experiências realizadas.

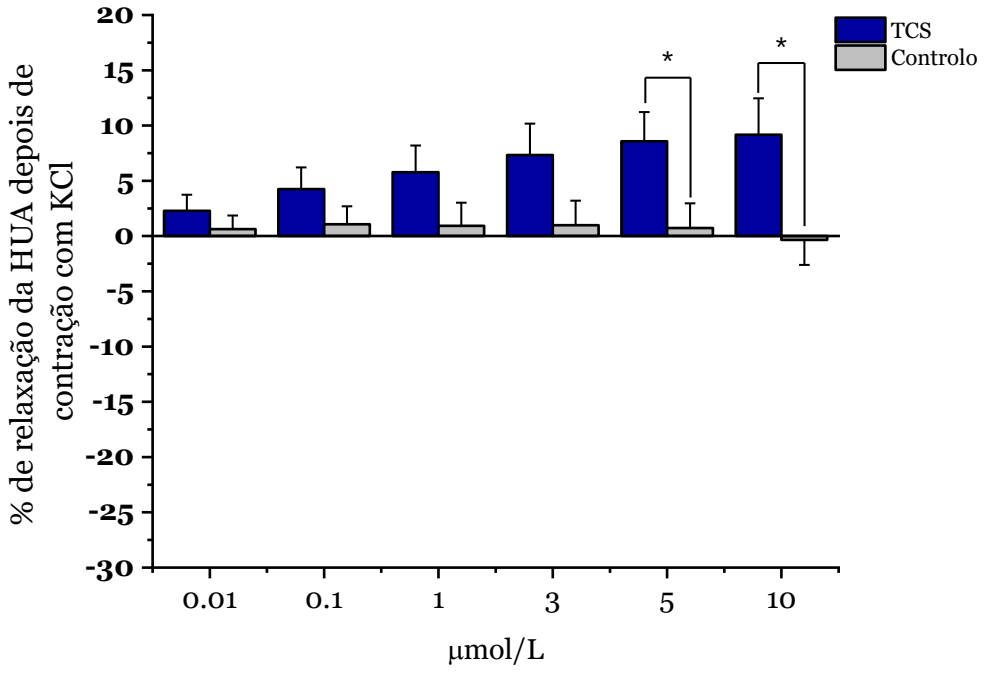


*Gráfico 2: Efeito do TCS (0,01; 0,1; 1; 3; 5 e 10 μM) sobre as contrações induzidas por cloreto de potássio (KCl, 60 mM) em HUA. Os dados foram expressos em percentagem (%) de relaxação sobre a contração provocada pela KCl. As barras representam a média e as linhas verticais o erro padrão da média (SEM). A análise estatística foi realizada pelo teste t-student, onde * P<0,05 versus o controlo e pelo teste one-way ANOVA com o teste post hoc Kruskal-Wallis, onde * P<0,05 versus concentrações de TCS.*

Como evidenciado no gráfico 2, diferenças estatisticamente significativas foram encontradas entre o grupo controlo (EtOH) e o TCS 5 e 10 μM ($p<0,05$, teste t-student). Verifica-se que apenas estas duas concentrações induzem relaxamento vascular

significativo. O efeito vasorelaxante do TCS para as concentrações de 5 e 10 μM (8,582 ± 2,638 % e 9,165 ± 3,296 %, respetivamente) não foi significativamente diferentes das outras concentrações de TCS (p=0,586, one-way ANOVA com teste post hoc Kruskal-Wallis).

4.1.3- Análise da exposição ao TCS na resposta contrátil

Anéis vasculares de artérias umbilicais humanas (HUA) incubadas durante 24 horas com TCS (10 e 1000 μM), foram contraídos utilizando dois agentes contrateis: serotonina (5-HT; 1 μM) e cloreto de potássio (KCl; 60 mM). Os dados previamente apresentados mostram que o TCS induz contração em artérias previamente contraídas com 5-HT. A tabela 5 mostra as diferenças significativas entre a contração com 5-HT e KCl nos grupos controlo (sem incubação), TCS 10 μM e TCS 1000 μM. Os resultados mostram que as incubações com TCS diminuem significativamente a contração provocada pela 5-HT.

*Tabela 5: Efeito contrátil da 5-HT e KCl no grupo controlo, TCS 10 μM e TCS 1000 μM. A análise foi feita a partir de uma two-way ANOVA com post hoc Holm-Sidak (*p<0,05).*

Agente contrátil	**Controlo (mg)**	**TCS 10 μM (mg)**	**TCS 1000 μM (mg)**
5-HT (1 μM)	3181,22 ± 279,48	2870,43 ± 258,75*	1998,35 ± 232,54*
KCl (60 mM)	2310,62 ± 328,86	2549,77 ± 232,54	2038,36 ± 252,80

4.1.4- Análise do envolvimento dos nucleótidos cíclicos no efeito induzido pelo TCS

De forma a analisar o envolvimento dos nucleótidos cíclicos, no efeito induzido pelo TCS, utilizou-se o nitroprussiato de sódio (SNP), um estimulador da guanil ciclase solúvel.

Os gráficos 3 e 4 resumem os resultados obtidos das várias experiências realizadas, para 5-HT e KCl, respetivamente.

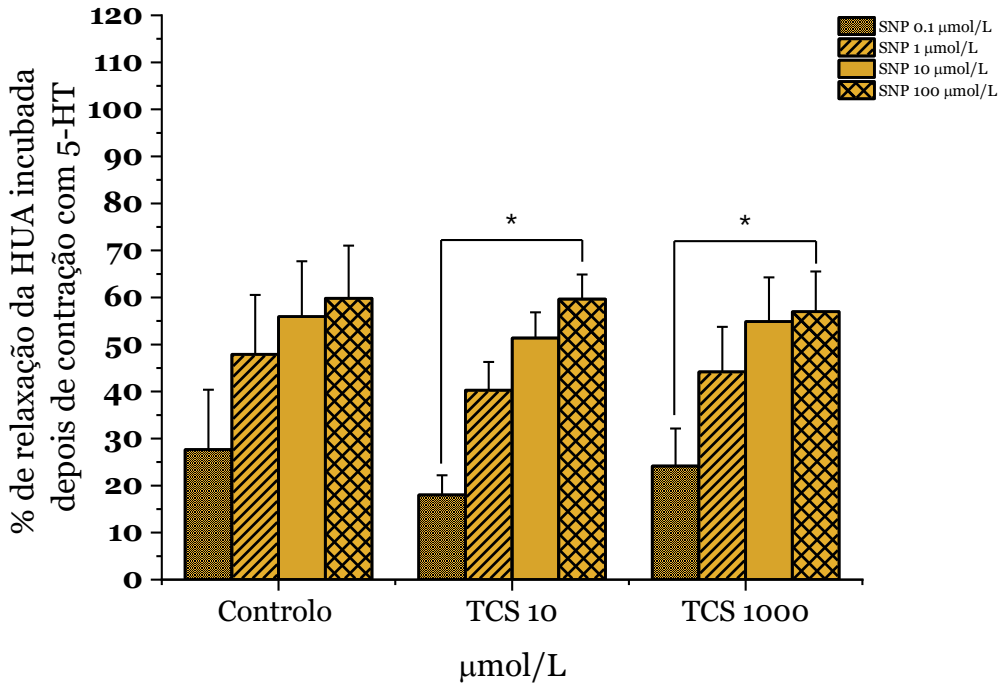


*Gráfico 3: Efeitos do nitroprussiato de sódio (SNP: 0,1; 1; 10 e 100 µM) sobre o efeito do triclosano (TCS; 10 e 1000 µM), em anéis de HUA contraídos com serotonina (5-HT; 1 µM). Os dados foram expressos em percentagem (%) de relaxação sobre a contração provocada pela 5-HT. As barras representam a média e as linhas verticais o erro padrão da média (SEM). A análise estatística foi realizada pelo teste two-way ANOVA, onde * P<0,05.*

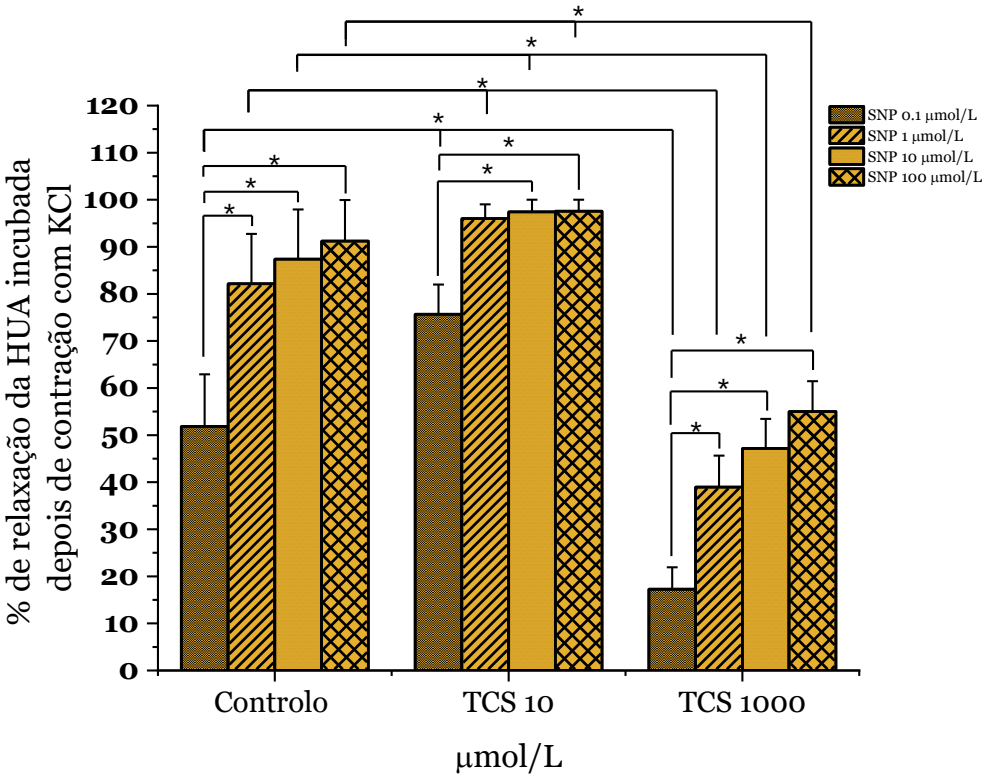


Gráfico 4: Efeitos do nitroprussiato de sódio (SNP: 0,1; 1; 10 e 100 µM) sobre o efeito do triclosano (TCS; 10 e 1000 µM), em anéis de HUA contraídos com cloreto de potássio (KCl; 60 mM). Os dados foram

*expressos em percentagem (%) de relaxação sobre a contração provocada pela KCl. As barras representam a média e as linhas verticais o erro padrão da média (SEM). A análise estatística foi realizada pelo teste two-way ANOVA, onde * P<0,05.*

Como apresentado nos gráficos 3 e 4 (referentes à 5-HT e KCl, respetivamente), diferenças estatisticamente significativas (* $p<0,05$) foram encontradas entre a concentração mais alta e mais baixa de SNP, nos grupos de TCS no gráfico 3, enquanto no gráfico 4 estas diferenças foram encontradas entre todos os grupos e as concentrações de SNP.

Em suma os dados obtidos referentes ao envolvimento dos nucleótidos cíclicos no efeito do TCS, demonstraram que o efeito conjunto do SNP e TCS é semelhante ao efeito do SNP sozinho para a 5-HT, no entanto na contração com KCl a concentração de TCS 10 μM potenciou o vasorelaxamento enquanto o TCS 1000 μM reduziu o relaxamento provocado pelo SNP.

4.1.5- Análise do envolvimento dos canais de cálcio no efeito induzido pelo TCS

De forma a analisar o envolvimento dos canais de cálcio, no efeito induzido pelo TCS, utilizou-se a nifedipina (Nif), um inibidor específico dos canais de cálcio do tipo L (LTCC).

Os gráficos 5 e 6 resumem os resultados obtidos das várias experiências realizadas, para 5-HT e KCl, respetivamente.

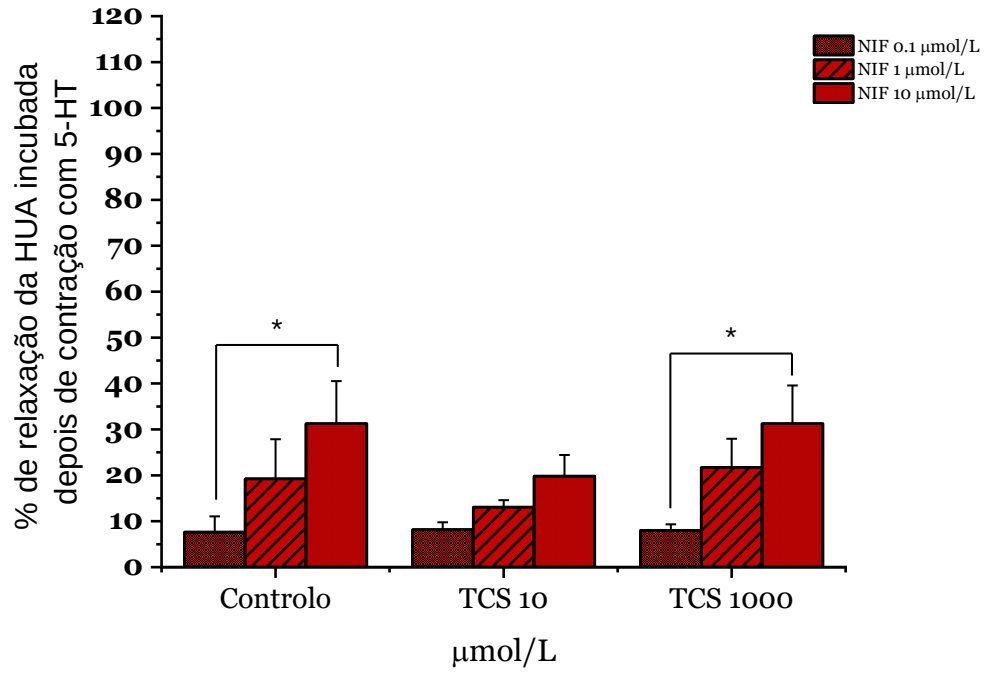


*Gráfico 5: Efeitos da nifedipina (Nif: 0,1; 1 e 10 µM) sobre o efeito do triclosano (TCS; 10 e 1000 µM), em anéis de HUA contraídos com serotonina (5-HT; 1 µM). Os dados foram expressos em percentagem (%) de relaxação sobre a contração provocada pela 5-HT. As barras representam a média e as linhas verticais o erro padrão da média (SEM). A análise estatística foi realizada pelo teste two-way ANOVA, onde * P<0,05.*

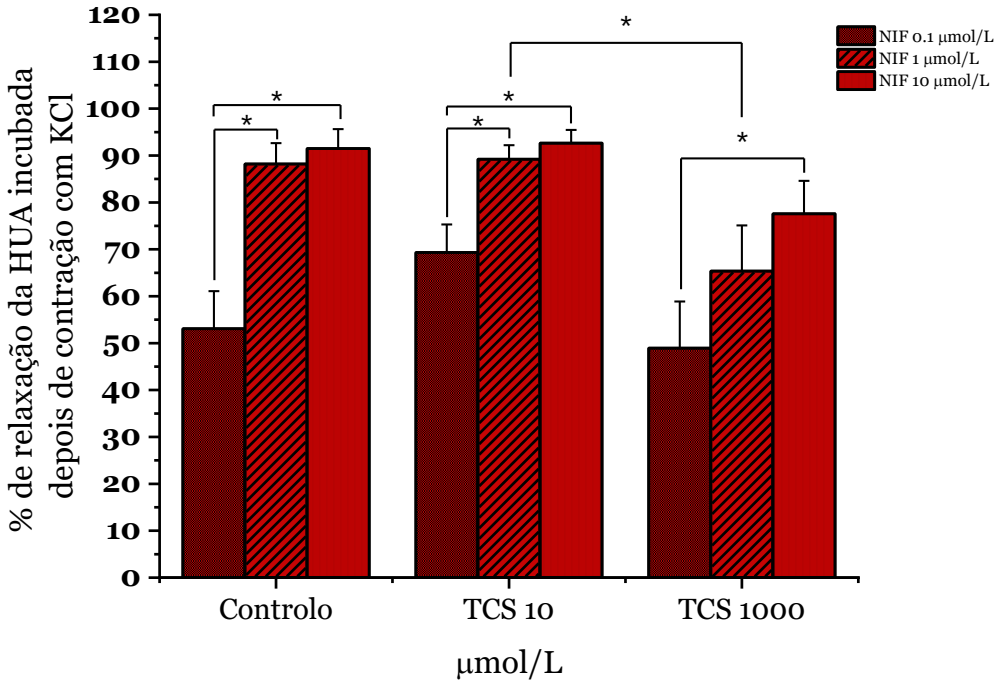


Gráfico 6: Efeitos da nifedipina (Nif: 0,1; 1 e 10 µM) sobre o efeito do triclosano (TCS; 10 e 1000 µM), em anéis de HUA contraídos com cloreto de potássio (KCl; 60 mM). Os dados foram expressos em percentagem (%) de relaxação sobre a contração provocada pela KCl. As barras representam a média e

*as linhas verticais o erro padrão da média (SEM). A análise estatística foi realizada pelo teste two-way ANOVA, onde * P<0,05.*

Como evidenciado nos gráficos 5 e 6 (referentes à 5-HT e KCl, respetivamente), não foram encontradas diferenças estatísticas entre o grupo controlo e os grupos de TCS, apenas para as concentrações mais alta e mais baixa no grupo controlo e no grupo TCS 1000 μM após contração com 5-HT, no entanto, houve diferenças significativas entre as concentrações de Nif de cada grupo para a contração com KCl ($p<0,05$).

Em suma, os dados obtidos referentes ao envolvimento dos canais de cálcio no efeito do TCS, demonstram que o efeito conjunto de Nif e TCS é semelhante ao efeito da Nif sozinha, para todos os agentes contrateis utilizados (5-HT e KCl).

Capítulo 5

Discussão

O triclosano (TCS) é um antibacteriano usado em produtos de higiene pessoal, desinfetantes e cosméticos [5]. As concentrações admitidas de TCS na União Europeia são 0,1-0,3% (m/m), no entanto apesar das concentrações máximas estarem bem definidas os efeitos do TCS não estão totalmente estudados de modo a saber se é seguro a estas concentrações [7,14,15]. Os estudos existentes sobre a análise dos efeitos do TCS a nível cardiovascular são maioritariamente focados em animais, sendo os principais efeitos observados a diminuição do output cardíaco e indução de vasorelaxamento em artéria aorta de rato [53,56]. Em humanos a quantidade de estudos é consideravelmente menor e focam-se maioritariamente em estudos *in vitro* com linhas celulares, em que o TCS impede a diferenciação em cardiomiocitos e leva à apoptose [65,66].

Tendo isto em conta o primeiro objetivo delineado foi a análise do efeito direto do TCS na HUA sem endotélio. Usando a técnica de Banho de órgãos estudou-se a contratilidade da HUA, em que o efeito de TCS (0,01; 0,1; 1; 3; 5 e 10 μM) foi analisado após contração com serotonina (5-HT, 1 μM) e cloreto de potássio (KCl, 60 mM). Contrações estáveis foram obtidas apos 10-15 minutos para os dois agentes contráteis, estando estes dados de acordo com Leung e colaboradores, que referem que as contrações provocadas pelo (KCl 60 mM) e pela 5-HT em HUA sem endotélio são semelhantes [83]. No entanto, alguns estudos também em HUA sem endotélio demonstram que as contrações provocadas pela 5-HT são superiores às contrações provocadas pelo KCl (60 mM) [84].

Relativamente ao efeito direto do TCS a nível vascular até à data ainda não foi realizado nenhum estudo de contratilidade vascular do TCS em humanos, sendo este um estudo pioneiro. No entanto o efeito do TCS (1, 3 e 5 μM) já foi testado em artéria aorta de rato onde o seu efeito era vasodilatador após contração com fenileferina (5 μM) e com uma solução fisiológica com elevada concentração de potássio (K^+) (60 mM) [53]. Os dados obtidos no presente trabalho demonstram que o TCS provoca um efeito vasoconstritor na HUA sem endotélio quando contraída previamente com 5-HT, o mesmo já não foi observado quando esta era contraída com KCl. Concordantemente, em células de aorta de rato contraídas com KCl o efeito do BPA (disruptor endócrino estruturalmente similar ao TCS) provocou relaxação com o aumento da concentração [85].

Devido a esta diferença de respostas houve a necessidade da realização de estudos mecanísticos. As artérias foram incubadas 24 horas com TCS (10 e 1000 μM) antes da

realização da técnica de Banho de órgãos. De seguida, a sua contratilidade foi analisada após contração com 5-HT (1 μM) e KCl (60 mM). Os resultados obtidos demonstram que existem diferenças significativas nas contrações induzidas por 5-HT, sendo que o TCS inibe a contração. Estes resultados coincidem com resultados previamente obtidos com outros disruptores endócrinos, em que a contração com 5-HT era diminuída quando as artérias eram incubadas com um disruptor endócrino [86,87].

Posteriormente foi usado o nitroprussiato de sódio (SNP) que é um estimulador da guanil ciclase solúvel. Os resultados obtidos mostram que o SNP relaxa as HUA contraídas por 5-HT e KCl, como já foi demonstrado em estudos anteriores realizados com HUA [81,88]. Quanto às artérias contraídas com KCl o TCS 1000 μM diminuiu o vasorelaxamento provocado pelo SNP enquanto o TCS 10 μM potenciou o vasorelaxamento. Nas artérias contraídas com 5-HT o TCS não influenciou o efeito do SNP. Estes dados voltam a ser coerentes com o efeito o BPA que, em artérias incubadas, diminui o relaxamento induzido pelo SNP [89]. Para além disso como previamente comprovado por Barberio e colaboradores, o BPA diminui a síntese de NO através de uma diminuição da expressão de óxido nítrico sintase endotelial (eNOS), recetor de estrogénio alfa (ERα) e recetor gamma ativado por proliferadores de peroxissoma (PPARγ) em artérias uterinas de ratos grávidas [90]. Alguns estudos demonstram a interação crosstalk que o TCS induz no recetor de hidrocarboneto de arilo (AhR), ER e PPARγ e afeta também a expressão das NOS [91-95], sendo assim poder se a assumir que a diminuição do vasorelaxamento induzido pelo SNP em artérias incubadas com TCS pode ser igualmente pela diminuição da síntese de NO.

Por outro lado, a modulação dos canais iónicos também é um dos principais efeitos das ações não genómicas dos estrogénios. Já foi descrito que a vasodilatação induzida pelos estrogénios pode ser devida a uma inibição dos canais de Ca^{2+} [96]. O cálcio extracelular pode entrar nas VSMC por diferentes tipos de canais de cálcio, como os LTCC, cuja inibição já foi associada à vasodilatação mediada pelos estrogénios [96,97].

Assim, o passo seguinte foi tentar perceber, se o efeito do TCS também pode ocorrer devido a uma ação do TCS nos canais de cálcio.

Para isso, a nifedipina (Nif), que é um inibidor dos canais de cálcio tipo-L, (LTCC) foi usada e o efeito do TCS (0,01-10 μM) analisado. Os dados obtidos mostram que a Nif relaxou as HUA contraídas tanto com 5-HT ou KCl. A relaxação induzida pela Nif foi também demonstrada em estudos anteriores realizados com HUA [98]. Os dados obtidos mostraram que o efeito da Nif (inibidor específico dos LTCC) nas artérias incubadas com TCS (10 e 1000 μM) foi similar às artérias controlo tanto na contração com 5-HT como

na contração com KCl. Assim, podemos concluir que o mecanismo do TCS não passa pela ativação LTCC.

Em suma, este estudo foi o primeiro a demonstrar os efeitos do TCS a nível vascular. Os dados demonstram que o TCS provoca vasoconstrição em artérias umbilicais humanas, e que essa vasoconstrição poderá ser devida à diminuição da síntese de NO pelas potenciais interações que o TCS pode ter com a eNOS, ER e PPARγ.

Conclusão

O TCS é um composto químico de utilização quotidiana, principalmente durante a pandemia COVID-19 onde a utilização de desinfetantes foi obrigatória para a entrada na maioria dos espaços comerciais. Contudo, estudos recentes demonstraram que este composto é um disruptor endócrino e que modula a atividade estrogénica e androgénica, podendo estar envolvido no aparecimento de patologias.

Neste sentido, este trabalho analisou pela primeira vez o mecanismo de ação do TCS na artéria umbilical humana, e foi demonstrado que este exerce um efeito arterial rápido (não genómicos), isto é, induz. O mecanismo de ação pode envolver a diminuição da síntese de NO pelas interações crosstalk que o TCS influencia nos ER, AhR e PPARγ, que por sua vez influenciam a atividade da eNOS. No entanto são necessários mais estudos para comprovar esta hipótese e melhor entender o mecanismo de ação do TCS a nível vascular.

Bibliografia

1. EC. Community Strategy for Endocrine Disrupters: a range of substances of interfering with the hormone systems of humans and wildlife. **1999**.
2. Guarnotta, V.; Amodei, R.; Frasca, F.; Aversa, A.; Giordano, C. Impact of Chemical Endocrine Disruptors and Hormone Modulators on the Endocrine System. *International Journal of Molecular Sciences* **2022**, *23*, 5710, doi:10.3390/ijms23105710.
3. Cooke, P.S.; Simon, L.; Denslow, N.D. Endocrine Disruptors. In *Haschek and Rousseaux's Handbook of Toxicologic Pathology*, Third edition ed.; 2013; pp. 1123-1154.
4. Kabir, E.R.; Rahman, M.S.; Rahman, I. A review on endocrine disruptors and their possible impacts on human health. *Environmental Toxicology and Pharmacology* **2015**, *40*, 241-258.
5. Pombo, M.; Castro-Feijoo, L. Endocrine disruptors. *Journal of Pediatric Endocrinology & Metabolism* **2005**, *18*, 1145-1155.
6. Dhillon, G.S.; Kaur, S.; Pulicharla, R.; Brar, S.K.; Cledon, M.; Verma, M.; Surampalli, R.Y. Triclosan: Current Status, Occurrence, Environmental Risks and Bioaccumulation Potential. *International Journal of Environmental Research and Public Health* **2015**, *12*, 5657-5684, doi:10.3390/ijerph120505657.
7. ECHA. Decision on substance evaluation pursuant to article 46(1) of regulation (EC) No 1907/2006. **2014**.
8. Axelstad, M.; Boberg, J.; Vinggaard, A.M.; Christiansen, S.; Hass, U. Triclosan exposure reduces thyroxine levels in pregnant and lactating rat dams and in directly exposed offspring. *Food and Chemical Toxicology* **2013**, *59*, 534-540, doi:10.1016/j.fct.2013.06.050.
9. Bedoux, G.; Roig, B.; Thomas, O.; Dupont, V.; Le Bot, B. Occurrence and toxicity of antimicrobial triclosan and by-products in the environment. *Environmental Science and Pollution Research* **2012**, *19*, 1044-1065, doi:10.1007/s11356-011-0632-z.
10. Shrestha, P.; Zhang, Y.M.; Chen, W.J.; Wong, T.Y. Triclosan: antimicrobial mechanisms, antibiotics interactions, clinical applications, and human health. *Journal of Environmental Science and Health Part C-Toxicology and Carcinogenesis* **2020**, *38*, 245-268, doi:10.1080/26896583.2020.1809286.
11. Weatherly, L.M.; Gosse, J.A. Triclosan exposure, transformation, and human health effects. *Journal of Toxicology and Environmental Health-Part B-Critical Reviews* **2017**, *20*, 447-469, doi:10.1080/10937404.2017.1399306.
12. Barbolt, T.A. Chemistry and Safety of Triclosan, and Its Use as an Antimicrobial Coating on Coated VICRYL* Plus Antibacterial Suture (Coated Polyglactin 910 Suture with Triclosan). **2002**, *3*, doi:10.1089/sur.2002.3.s1–45.
13. EC. Commission Regulation (EU) No 358/2014 amending Annexes II and V to Regulation (EC) No 1223/2009 of the European Parliament and of the Council on

cosmetic products. **2014**. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/ ?uri=celex%3A32014R0358 (accessed on 31 May 2022).

14. Infarmed. Análise Laboratorial de Produtos de Higiene Oral. **2017**. Available online: https://www.infarmed.pt/documents/15786/2215138/Produtos+de+Higiene+Oral/56215a29-68cb-48d5-b32f-650ecbcf0196 (accessed on 31 May 2022).
15. FDA. Safety and Effectiveness of Consumer Antiseptic Rubs; Topical Antimicrobial Drug Products for Over-the-Counter Human Use. **2019**, *Vol. 84*.
16. Bhargava, H.N.; Leonard, P.A. Triclosan: Applications and safety. *American Journal of Infection Control* **1996**, *24*, 209-218, doi:10.1016/s0196-6553(96)90017-6.
17. Olaniyan, L.W.B.; Mkwetshana, N.; Okoh, A.I. Triclosan in water, implications for human and environmental health. *Springerplus* **2016**, *5*, 17, doi:10.1186/s40064-016-3287-x.
18. SCCS. Scientific advice on the safety of Triclocarban and Triclosan as substances with potential endocrine disrupting properties in cosmetic products. **2022**. Available online: https://health.ec.europa.eu/publications/safety-triclocarban-and-triclosan-substances-potential-endocrine-disrupting-properties-cosmetic_en (Accessed on 31 May 2022)
19. Yueh, M.F.; Tukey, R.H. Triclosan: A Widespread Environmental Toxicant with Many Biological Effects. In *Annual Review of Pharmacology and Toxicology, Vol 56*, Insel, P.A., Ed.; Annual Review of Pharmacology and Toxicology; Annual Reviews: Palo Alto, 2016; Volume 56, pp. 251-272.
20. Allmyr, M.; Adolfsson-Erici, M.; McLachlan, M.S.; Sandborgh-Englund, G. Triclosan in plasma and milk from Swedish nursing mothers and their exposure via personal care products. *Science of the Total Environment* **2006**, *372*, 87-93, doi:10.1016/j.scitotenv.2006.08.007.
21. Wu, J.L.; Liu, J.; Cai, Z.W. Determination of triclosan metabolites by using in-source fragmentation from high-performance liquid chromatography/negative atmospheric pressure chemical ionization ion trap mass spectrometry. *Rapid Communications in Mass Spectrometry* **2010**, *24*, 1828-1834, doi:10.1002/rcm.4558.
22. Queckenberg, C.; Meins, J.; Wachall, B.; Doroshyenko, O.; Tomalik-Scharte, D.; Bastian, B.; Abdel-Tawab, M.; Fuhr, U. Absorption, Pharmacokinetics, and Safety of Triclosan after Dermal Administration. *Antimicrobial Agents and Chemotherapy* **2010**, *54*, 570-572, doi:10.1128/aac.00615-09.
23. Kanetoshi, A.; Ogawa, H.; Katsura, E.; Okui, T.; Kaneshima, H. DISPOSITION AND EXCRETION OF IRGASAN DP300 AND ITS CHLORINATED DERIVATIVES IN MICE. *Archives of Environmental Contamination and Toxicology* **1988**, *17*, 637-644, doi:10.1007/bf01055832.
24. Moss, T.; Howes, D.; Williams, F.M. Percutaneous penetration and dermal metabolism of triclosan (2,4,4 '-trichloro-2 '-hydroxydiphenyl ether). *Food and Chemical Toxicology* **2000**, *38*, 361-370, doi:10.1016/s0278-6915(99)00164-7.
25. Fang, J.L.; Vanlandingham, M.; da Costa, G.G.; Beland, F.A. Absorption and metabolism of triclosan after application to the skin of B6C3F1 mice. *Environmental Toxicology* **2016**, *31*, 609-623, doi:10.1002/tox.22074.

26. Geens, T.; Neels, H.; Covaci, A. Distribution of bisphenol-A, triclosan and n-nonylphenol in human adipose tissue, liver and brain. *Chemosphere* **2012**, *87*, 796-802, doi:10.1016/j.chemosphere.2012.01.002.
27. Fang, J.L.; Stingley, R.L.; Beland, F.A.; Harrouk, W.; Lumpkins, D.L.; Howard, P. Occurrence, Efficacy, Metabolism, and Toxicity of Triclosan. *Journal of Environmental Science and Health Part C-Environmental Carcinogenesis & Ecotoxicology Reviews* **2010**, *28*, 147-171, doi:10.1080/10590501.2010.504978.
28. Wu, Y.; Chitranshi, P.; Loukotková, L.; Gamboa Da Costa, G.; Beland, F.A.; Zhang, J.; Fang, J.-L. Cytochrome P450-mediated metabolism of triclosan attenuates its cytotoxicity in hepatic cells. *Archives of Toxicology* **2017**, *91*, 2405-2423, doi:10.1007/s00204-016-1893-6.
29. Zhu, L.Y.; Shao, Y.; Xiao, H.X.; Santiago-Schubel, B.; Meyer-Alert, H.; Schiwy, S.; Yin, D.Q.; Hollert, H.; Kuppers, S. Electrochemical simulation of triclosan metabolism and toxicological evaluation. *Science of the Total Environment* **2018**, *622*, 1193-1201, doi:10.1016/j.scitotenv.2017.11.317.
30. Rodricks, J.V.; Swenberg, J.A.; Borzelleca, J.F.; Maronpot, R.R.; Shipp, A.M. Triclosan: A critical review of the experimental data and development of margins of safety for consumer products. *Critical Reviews in Toxicology* **2010**, *40*, 422-484, doi:10.3109/10408441003667514.
31. Wang, L.Q.; Falany, C.N.; James, M.O. Triclosan as a substrate and inhibitor of 3 '-phosphoadenosine-5 '-phosphosulfate-sulfotransferase and UDP-glucuronosyl transferase in human liver fractions. *Drug Metabolism and Disposition* **2004**, *32*, 1162-1169, doi:10.1124/dmd.104.000273.
32. Provencher, G.; Berube, R.; Dumas, P.; Bienvenu, J.F.; Gaudreau, E.; Belanger, P.; Ayotte, P. Determination of bisphenol A, triclosan and their metabolites in human urine using isotope-dilution liquid chromatography-tandem mass spectrometry. *Journal of Chromatography A* **2014**, *1348*, 97-104, doi:10.1016/j.chroma.2014.04.072.
33. Sandborgh-Englund, G.; Adolfsson-Erici, M.; Odham, G.; Ekstrand, J. Pharmacokinetics of triclosan following oral ingestion in humans. *Journal of Toxicology and Environmental Health-Part a-Current Issues* **2006**, *69*, 1861-1873, doi:10.1080/15287390600631706.
34. Lorigo, M. Fetoplacental vasculature as a model to study human cardiovascular endocrine disruption. **2021**.
35. Lorigo, M.; Mariana, M.; Feiteiro, J.; Cairrao, E. How is the human umbilical artery regulated? *Journal of Obstetrics and Gynaecology Research* **2018**, *44*, 1193-1201, doi:10.1111/jog.13667.
36. Mesquita, I.; Lorigo, M.; Cairrao, E. Update about the disrupting ‐ effects of phthalates on the human reproductive system. *Molecular Reproduction and Development* **2021**, *88*, 650-672, doi:10.1002/mrd.23541.

37. Azzouz, A.; Rascon, A.J.; Ballesteros, E. Simultaneous determination of parabens, alkylphenols, phenylphenols, bisphenol A and triclosan in human urine, blood and breast milk by continuous solid-phase extraction and gas chromatography-mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis* **2016**, *119*, 16-26, doi:10.1016/j.jpba.2015.11.024.
38. Allmyr, M.; Harden, F.; Toms, L.M.L.; Mueller, J.F.; McLachlan, M.S.; Adolfsson-Erici, M.; Sandborgh-Englund, G. The influence of age and gender on triclosan concentrations in Australian human blood serum. *Science of the Total Environment* **2008**, *393*, 162-167, doi:10.1016/j.scitotenv.2007.12.006.
39. Calafat, A.M.; Ye, X.; Wong, L.Y.; Reidy, J.A.; Needham, L.L. Urinary concentrations of triclosan in the U.S. population: 2003-2004. *Environ Health Perspect* **2008**, *116*, 303-307, doi:10.1289/ehp.10768.
40. Jin, C.; Chen, Y.; Zhang, P.; Xiong, Z.; Wang, C.; Tian, Y. [Advances on research of human exposure to triclosan]. *Zhonghua Yu Fang Yi Xue Za Zhi* **2016**, *50*, 285-288, doi:10.3760/cma.j.issn.0253-9624.2016.03.021.
41. Macisaac, J.K.; Gerona, R.R.; Blanc, P.D.; Apatira, L.; Friesen, M.W.; Coppolino, M.; Janssen, S. Health Care Worker Exposures to the Antibacterial Agent Triclosan. *Journal of Occupational & Environmental Medicine* **2014**, *56*, 834-839, doi:10.1097/jom.0000000000000183.
42. Adolfsson-Erici, M.; Pettersson, M.; Parkkonen, J.; Sturve, J. Triclosan, a commonly used bactericide found in human milk and in the aquatic environment in Sweden. *Chemosphere* **2002**, *46*, 1485-1489, doi:10.1016/s0045-6535(01)00255-7.
43. Liu, M.; Ai, W.M.; Sun, L.M.; Fang, F.; Wang, X.D.; Chen, S.B.; Wang, H.L. Triclosan-induced liver injury in zebrafish (Danio rerio) via regulating MAPK/p53 signaling pathway. *Comparative Biochemistry and Physiology C-Toxicology & Pharmacology* **2019**, *222*, 108-117, doi:10.1016/j.cbpc.2019.04.016.
44. Yingjie, Q.; Jiayi, H.; Ping, H.; Jiangbo, Q.; Xubo, W.; Jun, W. Long-term exposure to environmental relevant triclosan induces reproductive toxicity on adult zebrafish and its potential mechanism. **2022**.
45. Montagnini, B.G.; Forcato, S.; Pernoncine, K.V.; Monteiro, M.C.; Pereira, M.R.F.; Costa, N.O.; Moreira, E.G.; Anselmo-Franci, J.A.; Gerardin, D.C.C. Developmental and Reproductive Outcomes in Male Rats Exposed to Triclosan: Two-Generation Study. *Front Endocrinol (Lausanne)* **2021**, *12*, 738980, doi:10.3389/fendo.2021.738980.
46. Kumar, V.; Chakraborty, A.; Kural, M.R.; Roy, P. Alteration of testicular steroidogenesis and histopathology of reproductive system in male rats treated with triclosan. *Reproductive Toxicology* **2009**, *27*, 177-185, doi:10.1016/j.reprotox.2008.12.002.
47. Stoker, T.E.; Gibson, E.K.; Zorrilla, L.M. Triclosan Exposure Modulates Estrogen-Dependent Responses in the Female Wistar Rat. *Toxicological Sciences* **2010**, *117*, 45-53, doi:10.1093/toxsci/kfq180.

48. Louis, G.W.; Hallinger, D.R.; Stoker, T.E. The effect of triclosan on the uterotrophic response to extended doses of ethinyl estradiol in the weanling rat. *Reproductive Toxicology* **2013**, *36*, 71-77, doi:10.1016/j.reprotox.2012.12.001.
49. Crawford, B.R.; deCatanzaro, D. Disruption of blastocyst implantation by triclosan in mice: Impacts of repeated and acute doses and combination with bisphenol-A. *Reproductive Toxicology* **2012**, *34*, 607-613, doi:10.1016/j.reprotox.2012.09.008.
50. Wang, X.L.; Chen, X.J.; Feng, X.J.; Chang, F.; Chen, M.J.; Xia, Y.K.; Chen, L. Triclosan causes spontaneous abortion accompanied by decline of estrogen sulfotransferase activity in humans and mice. *Scientific Reports* **2015**, *5*, 11, doi:10.1038/srep18252.
51. Feng, Y.X.; Zhang, P.; Zhang, Z.B.; Shi, J.C.; Jiao, Z.H.; Shao, B. Endocrine Disrupting Effects of Triclosan on the Placenta in Pregnant Rats. *Plos One* **2016**, *11*, 14, doi:10.1371/journal.pone.0154758.
52. Raj, S.; Sen Singh, S.; Singh, S.P.; Singh, P. Evaluation of Triclosan-induced reproductive impairments in the accessory reproductive organs and sperm indices in the mice. *Acta Histochemica* **2021**, *123*, 9, doi:10.1016/j.acthis.2021.151744.
53. Zhang, X.Y.; Zhang, X.Z.; Zhang, Y.Q.; Liu, M.Y.; Jin, J.; Yan, J.; Shen, X.; Hu, N.; Dong, D.L. Mitochondrial uncoupler triclosan induces vasorelaxation of rat arteries. *Acta Pharmaceutica Sinica B* **2017**, *7*, 623-629, doi:10.1016/j.apsb.2017.06.001.
54. Cherednichenko, G.; Zhang, R.; Bannister, R.A.; Timofeyev, V.; Li, N.; Fritsch, E.B.; Feng, W.; Barrientos, G.C.; Schebb, N.H.; Hammock, B.D.; et al. Triclosan impairs excitation-contraction coupling and Ca2+ dynamics in striated muscle. *Proc Natl Acad Sci U S A* **2012**, *109*, 14158-14163, doi:10.1073/pnas.1211314109.
55. Wang, D.; Zhang, Y.; Li, J.; Dahlgren, R.A.; Wang, X.; Huang, H.; Wang, H. Risk assessment of cardiotoxicity to zebrafish (Danio rerio) by environmental exposure to triclosan and its derivatives. *Environ Pollut* **2020**, *265*, 114995, doi:10.1016/j.envpol.2020.114995.
56. Saley, A.; Hess, M.; Miller, K.; Howard, D.; King-Heiden, T.C. Cardiac Toxicity of Triclosan in Developing Zebrafish. *Zebrafish* **2016**, *13*, 399-+, doi:10.1089/zeb.2016.1257.
57. Ma, Y.; Zang, L.X.; Wang, D.T.; Jiang, J.H.; Wang, C.H.; Wang, X.D.; Fang, F.; Wang, H.L. Effects of miR-181a-5p abnormal expression on zebrafish (Danio rerio) vascular development following triclosan exposure. *Chemosphere* **2019**, *223*, 523-535, doi:10.1016/j.chemosphere.2019.02.038.
58. Crofton, K.M.; Paul, K.B.; De Vito, M.J.; Hedge, J.M. Short-term in vivo exposure to the water contaminant triclosan: Evidence for disruption of thyroxine. *Environmental Toxicology and Pharmacology* **2007**, *24*, 194-197, doi:10.1016/j.etap.2007.04.008.
59. Zhang, P.; Yang, M.; Zeng, L.; Liu, C.J. P38/TRHr-Dependent Regulation of TPO in Thyroid Cells Contributes to the Hypothyroidism of Triclosan-Treated Rats. *Cellular Physiology and Biochemistry* **2018**, *45*, 1303-1315, doi:10.1159/000487558.
60. Taha, M.; Marie, A.M.; Ahmed-Farid, O.A. Combined approaches for evaluation of xenoestrogen neural toxicity and thyroid dysfunction: Screening of oxido-nitrosative

markers, DNA fragmentation, and biogenic amine degradation. *Journal of Biochemical and Molecular Toxicology* **2020**, *34*, 10, doi:10.1002/jbt.22521.

61. Wang, C.F.; Tian, Y. Reproductive endocrine-disrupting effects of triclosan: Population exposure, present evidence and potential mechanisms. *Environ Pollut* **2015**, *206*, 195-201, doi:10.1016/j.envpol.2015.07.001.
62. Zhang, N.; Wang, W.S.; Li, W.J.; Liu, C.; Chen, Y.Y.; Yang, Q.L.; Wang, Y.; Sun, K. Inhibition of 11 beta-HSD2 Expression by Triclosan via Induction of Apoptosis in Human Placental Syncytiotrophoblasts. *Journal of Clinical Endocrinology & Metabolism* **2015**, *100*, E542-E549, doi:10.1210/jc.2014-4376.
63. Yuan, G.X.; Ma, Y.; Zeng, Y.X.; Pan, H.B.; Liu, P.Y.; Liu, Y.; Liu, G.H.; Cheng, J.Q.; Guo, Y.S. Associations between low-dose triclosan exposure and semen quality in a Chinese population. *Environmental Pollution* **2022**, *299*, 7, doi:10.1016/j.envpol.2022.118926.
64. Jurewicz, J.; Wielgomas, B.; Radwan, M.; Karwacka, A.; Klimowska, A.; Dziewirska, E.; Korczak, K.; Zajdel, R.; Radwan, P.; Hanke, W. Triclosan exposure and ovarian reserve. *Reproductive Toxicology* **2019**, *89*, 168-172, doi:10.1016/j.reprotox.2019.07.086.
65. Zhang, M.; Zhu, R.R.; Zhang, L.B. Triclosan stimulates human vascular endothelial cell injury via repression of the PI3K/Akt/mTOR axis. *Chemosphere* **2020**, *241*, 7, doi:10.1016/j.chemosphere.2019.125077.
66. Du, G.Z.; Yu, M.M.; Wang, L.L.; Hu, W.Y.; Song, L.; Lu, C.C.; Wang, X.R. Transcriptome and DNA Methylome Dynamics during Triclosan- Induced Cardiomyocyte Differentiation Toxicity. *Stem Cells International* **2018**, *2018*, 8, doi:10.1155/2018/8608327.
67. Cullinan, M.P.; Palmer, J.E.; Carle, A.D.; West, M.J.; Westerman, B.; Seymour, G.J. The influence of a triclosan toothpaste on adverse events in patients with cardiovascular disease over 5-years. *Science of the Total Environment* **2015**, *508*, 546-552, doi:10.1016/j.scitotenv.2014.11.052.
68. Cullinan, M.P.; Palmer, J.E.; Faddy, M.J.; Westerman, B.; Carle, A.D.; West, M.J.; Seymour, G.J. The Influence of Triclosan on Biomarkers of Cardiovascular Risk in Patients in the Cardiovascular and Periodontal Study (CAPS): A Randomized Controlled Trial. *Journal of Periodontology* **2015**, *86*, 847-855, doi:10.1902/jop.2015.140716.
69. Braun, J.M.; Chen, A.M.; Hoofnagle, A.; Papandonatos, G.D.; Jackson-Browne, M.; Hauser, R.; Romano, M.E.; Karagas, M.R.; Yolton, K.; Zoeller, R.T.; et al. Associations of early life urinary triclosan concentrations with maternal, neonatal, and child thyroid hormone levels. *Hormones and Behavior* **2018**, *101*, 77-84, doi:10.1016/j.yhbeh.2017.11.009.
70. Cullinan, M.P.; Palmer, J.E.; Carle, A.D.; West, M.J.; Seymour, G.J. Long term use of triclosan toothpaste and thyroid function. *Science of the Total Environment* **2012**, *416*, 75-79, doi:10.1016/j.scitotenv.2011.11.063.
71. Berger, K.; Gunier, R.B.; Chevrier, J.; Calafat, A.M.; Ye, X.Y.; Eskenazi, B.; Harley, K.G. Associations of maternal exposure to triclosan, parabens, and other phenols with prenatal

maternal and neonatal thyroid hormone levels. *Environmental Research* **2018**, *165*, 379-386, doi:10.1016/j.envres.2018.05.005.

72. Sherer, D.M.; Al-Haddad, S.; Cheng, R.; Dalloul, M. Current Perspectives of Prenatal Sonography of Umbilical Cord Morphology. *International Journal of Womens Health* **2021**, *13*, 939-971, doi:10.2147/ijwh.s278747.
73. Martín, P.; Rebolledo, A.; Palomo, A.R.R.; Moncada, M.; Piccinini, L.; Milesi, V. Diversity of Potassium Channels in Human Umbilical Artery Smooth Muscle Cells: A Review of Their Roles in Human Umbilical Artery Contraction. *Reproductive Sciences* **2014**, *21*, 432-441, doi:10.1177/1933719113504468.
74. Tufan, H.; Ayan-Polat, B.; Tecder-Ünal, M.; Polat, G.; Kayhan, Z.; Ögüs, E. Contractile responses of the human umbilical artery to KCl and serotonin in Ca-free medium and the effeccts of levcromakalim. **2003**, *72* 1321-1329.
75. Santos-Silva, A.J.; Cairrao, E.; Verde, I. Study of the mechanisms regulating human umbilical artery contractility. *Health* **2010**, *02*, 321-331, doi:10.4236/health.2010.24049.
76. Izumi, H.; Makino, Y.; Shirakawa, K.; Garfield, R.E. Role of nitric oxide on vasorelaxation in human umbilical artery. *American Journal of Obstetrics and Gynecology* **1995**, 1478-1484.
77. Meyer, W.W.; Rumpelt, H.J.; Yao, A.C.; Lind, J. Structure and closure mechanism of the human umbilical artery. *European Journal of Pediatrics* **1978**, *128*, 247-259, doi:10.1007/bf00445610.
78. Nanaev, A.K.; Kohnen, G.; Milovanov, A.P.; Domogatsky, S.P.; Kaufmann, P. Stromal Differentiation and Architecture of the Human Umbilical Cord. *Placenta* **1997**, *18* 53-64.
79. Hurairah, H.; Ferro, A. The role of the endothelium in the control of vascular function. *International Journal of Clinical Practice* **2004**, *58*, 173-183, doi:10.1111/j.1368-5031.2004.0103.x.
80. Salemme, S.; Rebolledo, A.; Speroni, F.; Petruccelli, S.; Milesi, V. L, P-/Q- and T-type Ca²⁺ Channels in Smooth Muscle Cells from Human Umbilical Artery. *Cellular Physiology and Biochemistry* **2007**, *20*, 055-064, doi:10.1159/000104153.
81. Lovren, F.; Triggle, C. Nitric oxide and sodium nitroprusside-induced relaxation of the human umbilical artery. *British Journal of Pharmacology* **2000**, *131*, 521-529, doi:10.1038/sj.bjp.0703588.
82. Vanhoutte, P.M. Endothelium and control of vascular function. State of the Art lecture. *Hypertension* **1989**, *13*, 658-667, doi:10.1161/01.hyp.13.6.658.
83. Leung, S.W.S.; Quan, A.; Lao, T.T.; Man, R.Y.K. Efficacy of different vasodilators on human umbilical arterial smooth muscle under normal and reduced oxygen conditions. *Early Human Development* **2006**, *82*, 457-462.
84. Quan, A.; Leung, S.W.S.; Lao, T.T.; Man, R.Y.K. 5-hydroxytryptamine and thromboxane A2 as physiologic mediators of human umbilical artery closure. *Journal of the Society for Gynecologic Investigation* **2003**, 490-495.

85. Feiteiro, J.; Mariana, M.; Glória, S.; Cairrao, E. Inhibition of L-type calcium channels by Bisphenol A in rat aorta smooth muscle. *The Journal of Toxicological Sciences* **2018**, *43*, 579-586, doi:10.2131/jts.43.579.
86. Lorigo, M.; Quintaneiro, C.; Breitenfeld, L.; Cairrao, E. UV-B Filter Octylmethoxycinnamate Is a Modulator of the Serotonin and Histamine Receptors in Human Umbilical Arteries. *Biomedicines* **2022**, *10*, 1054, doi:10.3390/biomedicines10051054.
87. Lorigo, M.; Quintaneiro, C.; Breitenfeld, L.; Cairrao, E. UV-B Filter Octylmethoxycinnamate Alters the Vascular Contractility Patterns in Pregnant Women with Hypothyroidism. *Biomedicines* **2021**, *9*, 115, doi:10.3390/biomedicines9020115.
88. Santos-Silva, A.J.; Cairrão, E.; Morgado, M.; Álvarez, E.; Verde, I. PDE4 and PDE5 regulate cyclic nucleotides relaxing effects in human umbilical arteries. *European Journal of Pharmacology* **2008**, *582*, 102-109, doi:10.1016/j.ejphar.2007.12.017.
89. Gao, X.; Wang, H.-S. Impact of Bisphenol A on the Cardiovascular System — Epidemiological and Experimental Evidence and Molecular Mechanisms. *International Journal of Environmental Research and Public Health* **2014**, *11*, 8399-8413, doi:10.3390/ijerph110808399.
90. Barberio, L.; Paulesu, L.; Canesi, L.; Grasselli, E.; Mandalà, M. Bisphenol a Interferes with Uterine Artery Features and Impairs Rat Feto-Placental Growth. *International Journal of Molecular Sciences* **2021**, *22*, 6912, doi:10.3390/ijms22136912.
91. Szychowski, K.A.; Skóra, B.; Wójtowicz, A.K. Triclosan affects the expression of nitric oxide synthases (NOSs), peroxisome proliferator-activated receptor gamma (PPARγ), and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) in mouse neocortical neurons in vitro. *Toxicology in Vitro* **2021**.
92. Szychowski, K.A.; Wnuk, A.; Kajta, M.; Wójtowicz, A.K. Triclosan activates aryl hydrocarbon receptor (AhR)-dependent apoptosis and affects Cyp1a1 and Cyp1b1 expression in mouse neocortical neurons. *Environmental Research* **2016**, doi:http://dx.doi.org/10.1016/j.envres.2016.07.019.
93. Li, J.; Quan, X.; Zhang, Y.; Yu, T.; Lei, S.; Huang, Z.; Wang, Q.; Song, W.; Yang, X.; Xu, P. PPARγ Regulates Triclosan Induced Placental Dysfunction. *Cells* **2021**.
94. Ahn, K.C.; Zhao, B.; Chen, J.; Cherednichenko, G.; Sanmarti, E.; Denison, M.S.; Lasley, B.; Pessah, I.N.; Kultz, D.; Chang, D.P.Y.; et al. In vitro biologic activities of the antimicrobials triclocarban, its analogs, and triclosan in bioassay screens: Receptor-based bioassay screens. *Environmental Health Perspectives* **2008**, *116*, 1203-1210, doi:10.1289/ehp.11200.
95. Ohtake, F.; Baba, A.; Fujii-Kuriyama, Y.; Kato, S. Intrinsic AhR function underlies cross-talk of dioxins with sex hormone signalings. *Biochemical and Biophysical Research Communications* **2008**.
96. Cairrão, E.; Alvarez, E.; Carvas, J.M.; Santos-Silva, A.J.; Verde, I. Non-genomic vasorelaxant effects of 17β-estradiol and progesterone in rat aorta are mediated by L-type

Ca2+ current inhibition. *Acta Pharmacologica Sinica* **2012**, *33*, 615-624, doi:10.1038/aps.2012.4.

97. Okabe, K.; Inoue, Y.; Soeda, H. Estradiol inhibits Ca^{2+} and K^{+} channels in smooth muscle cells from pregnant rat myometrium. *European Journal of Pharmacology* **1999**, *376*, 101-108.
98. Saldanha, P.A.; Cairrão, E.; Maia, C.J.; Verde, I. Long- and short-term effects of androgens in human umbilical artery smooth muscle. *Clinical and Experimental Pharmacology and Physiology* **2013**, *40*, 181-189, doi:10.1111/1440-1681.12047.

Apêndice

https://www.mdpi.com/1422-0067/23/19/11427

International Journal of *Molecular Sciences*

MDPI

Review

Triclosan and Its Consequences on the Reproductive, Cardiovascular and Thyroid Levels

Ana C. Marques [1,2], Melissa Mariana [1,2] and Elisa Cairrao [1,2,*]

[1] Health Sciences Research Centre (CICS-UBI), University of Beira Interior, 6200-506 Covilhã, Portugal
[2] Faculty of Health Sciences (FCS-UBI), University of Beira Interior, 6200-506 Covilhã, Portugal
* Correspondence: ecairrao@fcsaude.ubi.pt; Tel.: +351-275-329-049

Abstract: Hygiene is essential to avoid diseases, and this is thanks to daily cleaning and disinfection habits. Currently, there are numerous commercial products containing antimicrobial agents, and although they are efficient in disinfecting, it is still not known the effect of the constant use of these products on human health. In fact, a massive use of disinfectants has been observed due to COVID-19, but the possible adverse effects are not yet known. Triclosan is one of the antimicrobial agents used in cosmetic products, toothpaste, and disinfectants. This compound is an endocrine disruptor, which means it can interfere with hormonal function, with its estrogenic and androgenic activity having already been stated. Even if the use of triclosan is well-regulated, with the maximum allowed concentration in the European Union of 0.3% (*m*/*m*), its effects on human health are still uncertain. Studies in animals and humans suggest the possibility of harmful health outcomes, particularly for the reproductive system, and in a less extent for the cardiovascular and thyroid functions. Thus, the purpose of this review was to analyse the possible implications of the massive use of triclosan, mainly on the reproductive and cardiovascular systems and on the thyroid function, both in animals and humans.

Keywords: triclosan; antimicrobial; endocrine disruptor; reproductive system; cardiovascular system; thyroid

Citation: Marques, A.C.; Mariana, M.; Cairrao, E. Triclosan and Its Consequences on the Reproductive, Cardiovascular and Thyroid Levels. *Int. J. Mol. Sci.* **2022**, *23*, 11427. https://doi.org/10.3390/ijms231911427

Academic Editors: Nicolas Chevalier and Charlotte Hinault-Boyer

Received: 9 September 2022
Accepted: 26 September 2022
Published: 28 September 2022

1. Introduction

Triclosan (TCS), 5-chloro-2-(2,4-dichloro phenoxy) phenol, is an endocrine-disrupting chemical often used as an antiseptic, disinfectant, or preservative [1–6].

An endocrine disruptor is a chemical substance that causes adverse effects in an organism by disrupting its endocrine function [7]. Many are chemicals present in the environment, which can be natural or synthetic and that can alter the endocrine functions by mimicking or inhibiting the action of endogenous hormones [8]. Exposure to these compounds can lead to alterations in one or more signalling pathways, that may induce negative effects on reproduction, growth, survival, behavioural, and obesity. These changes can be passed on to subsequent generations [9,10].

In the European Union, 85% of triclosan is used in personal care products (e.g., soaps, toothpaste, mouthwash, and cosmetics), and 10% in plastics and food contact materials (household utensils), while the remaining 5% is used in textiles (antibacterial clothing) [1–5,11]. It is also used as a suture coating, but only contributes 0.00016% of the overall TCS global exposure [12]. It has been used for these purposes since its introduction in 1968 [1]. Despite being used as a constituent of several products, there are still no defined regulations for TCS as it is considered tolerable and safe. Most personal care products contain between 0.1–0.3% (*w*/*w*) of triclosan [13]. As for its regulation, the European Commission and the Infarmed impose a maximum of 0.3% [6,14], while the FDA (U.S. Food and Drug Administration) has been decreasing the maximum allowed over the years [15].

Some studies presented by the European Chemicals Agency have concluded that triclosan can interfere with estrogenic (ER) and androgenic (AR) receptors and that it

has adverse effects on the thyroid and cardiovascular systems. Specifically, these studies have led to the discovery of the vasorelaxant properties of triclosan, of the effects that this compound may have in miscarriages or malformations of a foetus [16] and its potential to interfere with the thyroid hormones due to their structural similarity with TCS [17]. Besides the ERs and Ars, there are also other receptors that can be influenced by TCS. The aryl hydrocarbon receptor (AhR), a transcription factor that activates gene expression in a ligand-dependent manner, can modulate the function of oestrogen and androgen receptors, which means they are in crosstalk. TCS was previously tested with AhR because of its structural similarity to AhR-ligands, where it was discovered that TCS is a partial agonist of AhRs. Thus, it can be assumed that TCS agonistic activity toward AhR can influence ER and AR because of the crosstalk between these three receptors. A different receptor also affected by TCS is peroxisome proliferator-activated receptor gamma (PPARγ). This receptor is expressed in several reproductive tissues, as well as in rodent and human placentas, being involved in different metabolic processes. It was previously shown that PPARγ and AhR are in crosstalk since PPARγ agonists affect the expression of AhR and that AhR agonists affect PPARγ expression. Considering that TCS is a partial agonist of AhR and a weak agonist of PPARγ, it will affect the expression of both AhR and PPARγ. Thus, knowing that TCS interacts with all of these receptors, it is expected that TCS can influence the crosstalk between PPARγ, AhR, ERs and ARs [18–22].

Currently, the literature is still unclear about the potential effects of TCS on organisms and humans, and the impact on human health of the passive use of these compounds, mainly during the COVID-19 season, is still completely unknown. Therefore, and based on this assumption, the aim of this review was to analyse in more detail the possible implications of the massive use of triclosan, mainly on the reproductive and cardiovascular systems and on the thyroid function in both animals and humans.

2. Physical and Chemical Properties of Triclosan

Triclosan is a white, odourless, tasteless, crystalline powder with a molecular weight of 289.5 g/mol. It is commercially known as Irgasan DP 300 and Irgacare MP for oral applications [23,24].

Structurally, TCS is a molecule with functional groups for phenol ether and it has a lipophilic nature (log K_{ow} = 4.8), which suggests that it bioaccumulates in adipose tissue [25]. The chemical structure is a halogenated biphenyl ether which confers similar properties to many other toxicants, for example, polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), bisphenol A (BPA) and dioxins. It is a chlorinated phenoxy-phenol compound with a pKa of 8.1. It does not hydrolyse easily; however, the pH, the presence of metals and dissolved organic matter can affect the photosensitivity of triclosan [24] (Table 1).

It is almost insoluble in water, relatively soluble in alkaline solutions and soluble in most non-polar organic solvents (Table 2).

Moreover, TCS also has impurities associated with it such as copper, mercury, lead, and cadmium, among others [6].

Table 1. Triclosan general properties [26–29].

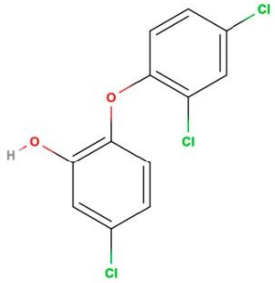
CAS No.	3380-34-5
Chemical structure	
Molecular formula	$C_{12}H_7Cl_3O_2$
Commercial name	Irgasan DP 300, FAT 80′023, CH 3565, GP41-353, Irgacare MP, Lexol 300, Cloxifenolum e Ster-Zac
Form	Powder or crystalline powder
Colour	White
Applications	Antimicrobial, antiseptic, and preservative
Nature	Hydrophobic/lipophilic
Molecular weight	289.5 g/mol
Density	1.49 g/cm^3
Dissociation constant (pKa) (20 °C)	7.9
Henry constant (Hc) (atm mol^{-1}·m^{-3})	1.5×10^{-7} (25 °C)
Octanol-water partition coefficient (log K_{ow})	4.8
Sorption coefficient (K_{oc})	18,408
Vapour pressure	4×10^{-6} Pa (mm Hg a 20 °C)
Triclosan degradation products	Methyl-TCS, dioxins, chlorophenol, chloroform

Table 2. Solubility of triclosan in different solvents [27,28].

Solvent	**Solubilities at 25 °C (g Triclosan/100 g Solvent)**
Distilled water (20 °C)	0.001
Acetone	>100
Ethanol 70% or 95%	>100
Isopropanol	>100
Propylene glycol	>100
Hexane	8.5
Tween 20	>100
Glycerine	0.15

It is a broad-spectrum antimicrobial agent whose activity depends on its concentration and formulation, that is, at low concentrations, TCS is bacteriostatic while at high concentrations it is bactericidal. This antibacterial effect has been widely studied and was demonstrated to be related with the inhibition of a specific enzyme in fatty acids biosynthesis, the enoyl-acyl carrier protein reductase (ENR). This inhibition will suppress the

membrane phospholipids and the proliferation of microbial cells. TCS has been considered safe for humans and animals since ENR is absent in eukaryotes; however recent studies demonstrate that TCS is considered toxic for eukaryotes [30,31]. It acts primarily on the cytoplasmic membrane and its entry into the cells appears to be by diffusion [6,23,32,33] due to the lipophilic properties of TCS. These allow TCS to be incorporated into the phospholipid bilayer, which will disturb phospholipid packing resulting in the formation of lipid pores and alterations in the membrane permeability to ions and larger molecules. This mechanism is known as the TCS membranotropic effect. In 2003, Lygre et al. demonstrated that this was the mode of action by which TCS exerts its toxic effect on eukaryotes, destabilizing the membrane of a cell or the mitochondria [30,31,34].

This compound has a half-life of approximately 11 days at the water surface and is degraded in aerobic soil with a half-life of 18 days [2].

3. Metabolism

TCS can be absorbed through several routes, including the mucous membrane of the oral cavity, dermal exposure, or the gastrointestinal tract. Considering that most products containing TCS are used for dermal application, its main route of absorption is the skin, through which it is rapidly absorbed due to its lipophilic properties [35] (Table 3). Additionally, Wu et al. noted that TCS can also be absorbed orally which leads to the detection of metabolites in plasma, and also through the gastrointestinal tract [32].

Table 3. Concentration of triclosan in personal care products.

Type of Product and Category	Triclosan Concentration (%)	References
Oral hygiene		
Toothpaste	0.3	[6]
Mouthwash	0.03	[36]
Skin washing products		
Liquid soap	0.1 to 0.45	[37]
Shower gel	0.3	[28]
Dishwasher	0.1	[3]
Products applied to the skin		
Body lotion	0.3	[28]
Facial moisturizer	0.3	
Deodorant	0.3	

Concentrations in the millimolar range have been detected in humans; therefore, considering the amount of TCS found in human consumer products, all exposure routes are important for the final exposure [38]. In a study presented in 1992 [39] when triclosan (1.6 mg) was applied directly to the skin of rats, it was rapidly absorbed and the concentration peak occurred between 12 and 18 h following exposure. Further to this study, Moss, Howes and Williams demonstrated that triclosan penetrates faster through rat skin than through human skin and that after 24 h only about 12% of the dose was on human skin and about 26% on rat skin [40].

Regarding the distribution of this substance, the studies currently available only concern rodents. Kanetoshi et al. were the first to demonstrate the metabolization of this substance in the bile and the liver. The authors demonstrated that within 24 h of TCS administration, the highest levels were present in the bile (33.6 µg TCS/g tissue), followed by the liver which contained about 3.0 µg TCS/g tissue. Concentrations in these two tissues remained high for 24 h after administration when compared to other tissues [39].

In another study performed using rats, this compound was detected in the 15 tissues analysed 12 h after a skin application, with the gallbladder and bladder containing the highest

concentrations while the testes, thymus and brain had the lowest concentrations [41]. Similarly, Geens et al. extracted adipose tissue, brain tissue and liver from eight men and three women (aged between 9 and 64 years) by autopsy, and measured TCS. These authors concluded that the liver and adipose tissue were better retainers of TCS than brain tissue [42].

Regarding metabolism itself, TCS can be metabolised by three pathways: cytochrome P450 (P450), UDP-glucoronyltransferase (UGT) and sulphotransferase (SULT). The latter two are part of the phase II metabolism of xenobiotics [26,43].

The main metabolite of TCS hydroxylation is triclosan monohydroxylate. This in turn can be cleaved to 2,4-dichlorophenol and 4-chlorocatechol. A fourth metabolite can also be formed from the hydroxylation of 2,4-dichlorophenol, forming 3,5-dichlorocatechol [44] (Figure 1).

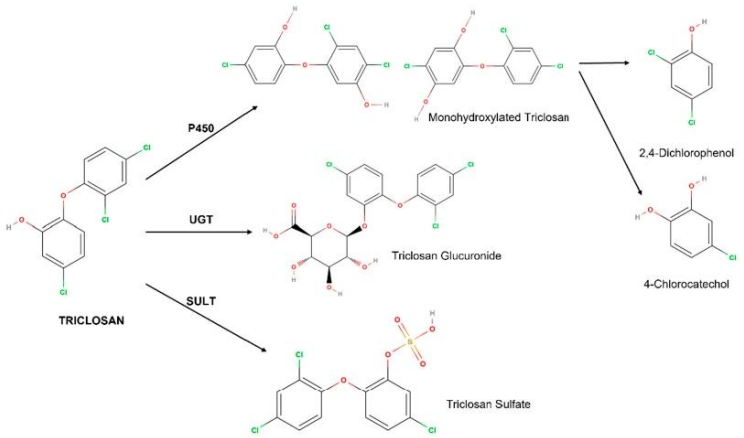


Figure 1. Metabolism of triclosan and its metabolites.

The fact that there are two pathways of the phase II metabolism is not influenced by the route of administration; however, the ratio between the transformation of TCS by the two pathways is already influenced by the species [25,45].

Glucuronidation and the sulphation of TCS causes the addition of glucuronic acid and sulphate, respectively, to the hydroxyl group, thus destroying the proton translocating nature and adding a charged/polar group to the TCS. This in turn will enhance its hydrophilic properties and decrease TCS's ability to accumulate in adipose tissue [3].

The liver is the main site of TCS metabolism; however, it also occurs at minimal levels in the skin. It is primarily metabolized through the SULT pathway, yet 24 h after administration, both phase II metabolites are found. Despite undergoing metabolization, the unmetabolized form of TCS remains the predominant form [40].

In the liver, the production of metabolites depends on the dose of TCS. For exposures of 1–5 µM of TCS for 30 min, there was an equal production of TCS-sulphate and TCS-glucuronyl, and for exposures greater than 20 µM the glucuronidation predominated while for doses below 1 µM it led to sulphation [46].

In a 2014 study, 46 volunteers, namely, 26 men and 20 women aged between 4 and 80 years, provided urine samples which were tested and both TCS-sulphate and TCS-glucuronyl were detected. The main metabolite identified was TCS-glucuronyl [47].

Regarding the elimination of TCS, the main route of excretion is through urine (57–87% of an administered dose) and faeces is the secondary route (5–33% of an administered dose). This compound is excreted mainly in its metabolised forms: TCS-glucuronic and TCS-sulphated; thus, during the first 4 days after an exposure, between 24% and 83% of the TCS consumed is excreted, and only after 8 days does it reach basal excretion levels [3,45,48].

Triclosan has already been detected in several matrices, such as in urine, blood, serum, and breast milk (Table 4). For this reason, some studies have been conducted to determine whether or not the presence of TCS can affect the foetus, through crossing the placental barrier, or even if it can induce adverse effects in future generations, as already observed for other endocrine disruptors [49–51].

Table 4. TCS concentrations in biological fluids.

Fluid	Concentration (nM)	Country	Reference
Serum	4.1–41.4	Spain	[52]
Plasma	0.0035–1200	Australia, Sweden	[35,53]
Urine	8.3–13090	USA	[53,54]
	0.56 ± 1.8 (non-obese)	India	[55]
	0.16 ± 0.27 (obese)		
	1.1–7.3	Spain	[52]
	0.51 ± 0.53	USA	[56]
Breast milk	0.86–7.3	Spain	[52]
	0.062–252	USA, Australia, Sweden	[33,53]

4. Effects on the Reproductive System

4.1. Animals

TCS has been widely studied in animals at the level of the reproductive system. It is also known that in this system, the effects are mainly due to the modulation of androgenic and estrogenic receptors.

In a study conducted by Ishibashi et al., several concentrations of triclosan were tested on embryos and adults of the *Oryzias latipes* fish. At concentrations of 625, 1250 and 2500 µg/L, no embryos were born, and they found that at 313 µg/L the incubation time increased significantly; however, fertility was not affected during the study [57].

Liu et al. used zebrafish (*Danio rerio*) to test the toxic effects of three concentrations of TCS (0.08, 0.16 and 0.25 mg/L) on the liver. The growth of the zebrafish showed no significant changes; however, there was an increase of 11.67–19.16% in the zebrafish weights and of 21.53–47.42% in the liver weights at exposures of 0.16 and 0.25 mg/L. At these concentrations, the superoxide dismutase (SOD) activity decreased while the malondialdehyde (MAD) levels gradually increased with different TCS concentrations. Thus, the authors demonstrated that exposure to TCS causes oxidative stress in the liver depending on the exposure time and concentration. This exposure created several hepatocellular changes, an increased hepatic plaque gap, necrosis, and atrophy, with the last two being concentration dependent. The same authors also observed a TCS concentration-dependent apoptosis and they quantified the expression of Bcl-2 and Bax to better understand which pathway of apoptosis was promoted by TCS. The expression of Bcl-2 at concentrations of 0.16 and 0.25 mg/L was significantly decreased while Bax was increased at concentrations of 0.08 and 0.25 mg/L. This relationship between Bax and Bcl-2 led the authors to hypothesise that hepatocyte apoptosis is directly related to the exposure of zebrafish to TCS [58].

Recently, Qiao et al. also tested 2, 20 and 200 µg/L of TCS in zebrafish, observing no mortality or any abnormality in their behaviour during the experiment. Fertilisation was not affected at any of the concentrations; however, the births decreased. Furthermore, in males, the testosterone levels decreased with the higher TCS concentration while the oestradiol and vitellogenin (VTG) levels increased in the 20 and 200 µg/L of TCS groups. On the other hand, in females, there was only a decrease in the VTG levels at 20 and 200 µg/L of TCS. These results indicate that TCS has an estrogenic influence in zebrafish, with males being more sensitive than females. There was also a significant decrease in mature spermatozoa and destruction of the testis structure at the concentration of 200 µg/L

of TCS, and in females, an increase in the number of immature oocytes was observed at the same concentration [59].

Another model widely used for in vivo studies are rats. Kumar et al. observed that the administration of 5 mg/kg/day to male Wistar rats did not induce a significant change in the testes weight, but higher doses of TCS induced a significant change in the testes and sexual tissues. The weight of the testes, epididymis, ventral prostate, vas deferens and seminal vesicles decreased by 20–50% with 10 mg/kg/day and 35–49% with 20 mg/kg/day. The authors also quantified several hormones and observed a decrease of 38.5% in the luteinizing hormone (LH), 17% in the follicle stimulating hormone (FSH), 35% in cholesterol, 31% in pregnenolone and 41% in testosterone after a dose of 20 mg/kg/day [60].

Regarding the study of gender differences in reproduction, in the following year, 2010, Stoker et al. performed a daily administration of TCS (9.375; 37.5; 75; 150 mg/kg) to female Wistar rats, between the Postnatal day (PND) 22 and the PND 42 (PND = 0, day of birth of the females used in the study), to examine the effects of TCS on female pubertal development. They analysed if the time until the opening of the vaginal canal was affected, as well as the histology of the uterus and ovaries, and some hormones including E2 and LH. The authors observed that this compound altered the reproductive development and response to exogenous oestrogens. They also observed a lower age for the opening of the vaginal canal with the administration of 150 mg/kg, which was an estrogenic response of the triclosan since stimulation through oestrogens is required for the canal to open. The serum oestradiol concentrations at PND 42 were decreased followed by administration of TCS at 37.5 mg/kg and 150 mg/kg, while the LH levels did not show significant changes. Thus, the study suggested that TCS increases oestrogen activity with a potential to alter the oestrogen-dependent functions. In summary, the authors concluded that TCS affects the reproductive development and uterine responses to exogenous oestrogens in developing females [61].

Two years later, in 2012, Jung et al. analysed the estrogenic activity of TCS using well-established models, both in vivo and in vitro. For the in vivo approach, female Sprague-Dawley rats were treated with 17α-ethinyl-oestradiol (1 mg/kg) and TCS (7.5, 37.5 and 187.5 mg/kg), between PND 19 and 21. The TCS increased the uterine weight and mRNA expression for complement 3, which is an oestrogen-sensitive gene in the uterus, in immature mice after a treatment with TCS at 37.5 mg/kg and 187.5 mg/kg. This shows that TCS has estrogenic activity. In vitro, the estrogenic activity upon TCS exposure was analysed on the expression of calbindin-D9k (CaBP-9k), which is a biomarker for the detection of endocrine disruptors in GH3 cells (i.e., Wistar rat epithelial cells). The expression of CaBP-9k was increased in the presence of TCS, which demonstrates the endocrine disrupting nature of TCS [62]. In a similar study by Louis et al., also using female Wistar rats between PND 19 and 21, doses of ethinyl-oestradiol (0.125, 0.25, 0.5, 1, 2 and 3 μg/kg) or ethinyl-oestradiol combined with TCS (2.3, 4.69, 9.375 and 18.75 mg/kg) were administered orally. No changes in the body weight were observed; however the uterine weight was increased when ethinyl-oestradiol (1, 2 and 3 μg/kg) was combined with TCS (4.69, 9.375 and 18.75 mg/kg). The expression of CaBP-9K was also analysed, but they found no changes when treated with TCS alone; however, the coupling of ethinyl-oestradiol and TCS caused an increase in its expression, but not significantly higher than the expression increase with the ethinyl-oestradiol alone. The authors concluded that TCS can alter the uterine response in the presence of low concentrations of ethinyl-oestradiol [63].

Regarding the effects of TCS in *Mus musculus* rats' gestation, Crawford et al. compared several doses of TCS (0, 87, 262, 523 and 785 mg/kg) with BPA. Females receiving the TCS dose on gestational days 0 and 1 showed no significant differences in the implantation sites when compared with the control. On gestational day 2, significant differences were already observed for the 785 mg/kg triclosan dose and on day 3 for the 523 mg/kg triclosan dose. When exposed to 18 and 785 mg/kg of triclosan from gestational days 1–3, the number of implantation sites decreased significantly on gestational day 6. The number of implantation sites was also reduced after an injection of these two doses on gestational day 3 and only of

the higher dose on gestational day 2 [64]. Thus, this study concluded that TCS may affect intrauterine implantation when administered in combination with BPA.

In a different study, 3-month-old rats were administered with 1, 10 and 100 mg TCS/kg/day from gestational day 6. The TCS levels were measured up to gestational days 11 and 16. An exposure to TCS demonstrated a dose-dependency in increasing foetal death. The incidence of spontaneous abortion at doses 10 and 100 reached 60% and 80%, respectively. The level of oestrogen sulphotransferase protein (EST) was decreased at gestational day 16 at the highest dose, as were its plasma and placental activities. These abortions were most likely caused by the inhibition of EST activity leading to placental thrombosis and degeneration [65]. Thus, the authors concluded that higher levels of TCS may lead to the occurrence of spontaneous abortions due to the inhibition of EST activity.

One year later, in 2016, Feng et al. recorded the total and uterine weights of female mice throughout gestation after an administration of TCS (0, 30, 100 and 300 mg/kg), and noted a lower weight gain in the groups where 300 and 600 mg/kg of TCS was administered when compared to the control; however, significant differences were only found regarding the uterine weight in the 600 mg/kg group. The tissues presenting the highest concentration of TCS were the placenta, liver, and kidneys with 12.83, 9.52 and 8.74 µg/g, respectively. In the placenta, the mean TCS levels following 0, 30, 100 and 300 mg/kg of TCS administrations were 0.033, 13.05, 28.23 and 55.83 µg/g, respectively. The LH and FSH levels were identical to the control group, whereas the levels of human chorionic gonadotropin (hCG), prolactin, progesterone, oestradiol, and testosterone were significantly reduced in all the exposure groups when compared to the control. Thus, the placenta may be a target of TCS in rats during the gestation period [66].

In the same way, a study performed by Montagnini et al. tested the effect of low doses of TCS (0.8, 2.4, and 8.0 mg/kg) over the course of three generations of Wistar rats named F0, F1 (the offspring of F0) and F2 (the offspring of F1). The TCS was orally administered to the F0 generation only between PND 49 and 120 in females, while in males it continued until PND 140. Thus, weight changes, food consumption during the pre-mating period, sexual behaviour assessment, male organs of the F1 generation, plasma testosterone quantification, all sperm parameters, testicular histomorphometry, sexual and physical development of the F1 and F2 generations and neuronal behavioural tests were accounted for. They concluded that these concentrations of TCS had no significant effect other than a reduction in the viability and motility of spermatozoa from the F1 generation being administered with 2.4 mg/Kg at PND 140. Doses administered between PND 49 and PND 140 did not affect the sperm parameters; therefore, the authors concluded that TCS could have an impact on gametogenesis during the foetal period [67].

More recently, Raj et al. analysed the effects of accumulated TCS on the histopathology and secretory functions of the epididymis, seminal vesicle, and sperm indices in adult Swiss-strain rats. Four different doses of triclosan (40, 80, 160 and 320 mg/kg) were administered for 42 consecutive days. All the concentrations significantly decreased the epididymal and seminal vesicle weights and led to a decrease in the sperm percentage and viability, and sperm count; furthermore, the percentage of non-viable spermatozoa increased. The histology of the epididymis was marked with changes throughout its length with disorganization and the appearance of vacuoles. The final chromatographic study showed an accumulation of TCS in the epididymis and seminal vesicle. Thus, Raj et al. concluded that TCS caused alterations in the epididymis and seminal vesicle, as well as in the sperm indices, epididymal sialic acid levels and fructose levels of the seminal vesicle [68].

Concerning the effect of this compound on the placenta, in 2010, TCS was tested on the placental tissue from ewes with gestation periods ranging between 126–130 days. In this study it was demonstrated that the TCS was a potent inhibitor of placental EST activity. This inhibition was analysed, and the authors observed that it occurred mainly competitively; therefore, it can be assumed that TCS occupies the substrate binding sites. The role of placental EST is not yet fully known; however, alterations in its expression are

thought to be associated with spontaneous abortions and foetal loss [69], as previously demonstrated in rats by Wang et al. [65].

In summary, TCS has a variety of reproductive effects in fish, rats and even sheep. The decreased fertility beyond the induction of abortion was the most observed consequence in any of the models. This may be due to the way the substance acts on the receptors or on oestradiol concentrations, as observed by Wang et al., where they demonstrated an inhibition of EST activity which caused a thrombosis in the placenta. Furthermore, TCS also appears to affect the serum concentration of several hormones such as LH, FSH, hCG, pregnenolone and cholesterol, which can lead to reproductive problems such as foetal loss and decreased fertility in subsequent generations.

4.2. Humans

In humans, TCS has not been widely studied yet, with most of the existing studies being related to the hormonal levels of EST, FSH and the effects at the foetal-placental level, namely, spontaneous abortions.

The first authors to demonstrate that TCS could be associated with adverse reproductive effects were Wang et al., finding an association between urinary TCS concentration levels and increased spontaneous abortions. These authors recruited a population of 452 women at 14 and 24 weeks of pregnancy with mean ages of 28 and 27 years, respectively. Their E2 levels at low-TCS concentrations were lower than the control; however, at high-TCS levels there were no changes. The plasma EST levels were reduced at both low- and high-TCS concentrations, while the EST activity at high-TCS concentrations was lower compared to the control and the low-TCS concentrations [70]. This increase in the number of abortions at higher TCS concentrations may be due to decreased EST activity caused by the TCS, as it was already demonstrated in animals.

Miscarriages may be linked to an inhibition of the placental 11β-HSD2 enzyme. In this sense, Zhang et al. observed that triclosan altered the expression of 11β-HSD2 in human syncytiotrophoblasts and examined the apoptotic mechanism induced by TCS. At concentrations of 0.1 μM and higher of TCS, the mRNA levels decreased significantly, and at the protein level there was a significant inhibition over a similar range of concentrations. There was also a decrease in the viability of syncytiotrophoblasts in a concentration dependent manner (0.001–10 μM), with statistical significance at concentrations above 0.1 μM. Moreover, the authors observed that triclosan induced the apoptosis of syncytiotrophoblasts and that the inhibition of 11β-HSD2 was explained by the induction of apoptosis [71].

In 2017, an epidemiological study also demonstrated the association between the urinary TCS levels of 401 pregnant women (at 16 weeks of gestation) and birth weight, length, head circumference and length of gestation. Urine was collected at 16 and 27 weeks of gestation, and TCS was detected in 91% and 83% of the samples, respectively, with a mean concentration of 16 ng/mL. This study showed a positive association between the urine triclosan concentration and a moderate reduction in birth weight, length, head circumference and gestational length [72].

Two years later, Jurewicz et al. analysed the association between triclosan concentration and ovarian reserve, which is a marker of female fertility. They counted the antral follicles and quantified the anti-müllerian hormone, follicle stimulating hormone and oestradiol. The authors recruited 511 menstruating women (25–39 years old) that provided blood and urine samples. The blood samples were taken at the beginning of the menstrual cycle, approximately between the second and fourth day, so that the hormones could be counted, while the antral follicle counts were performed in both ovaries during the beginning of the follicular phase of the menstrual cycle. Urine TCS concentrations (0.3–1677.68 ng/mL) were shown to be negatively associated with the antral follicle count, while the anti-müllerian hormone, follicle stimulating hormone and oestradiol did not show a statistic relevance. Thus, the authors concluded that TCS may be associated with decreased female fertility, although further studies are needed to demonstrate the mechanism involved in this effect [73].

The following year, Yuan et al. compared the levels of TCS present in urine with the sperm quality in a Chinese population, between 2018 and 2019. From the 406 subjects (aged between 21 and 56), 57.1% had never smoked and 83.5% had never consumed alcoholic beverages. The parameters analysed included the sperm volume, concentration, count, total, progressive and non-progressive motility, immobility, percentage of hyperactivated sperm, mean velocity, curvilinear velocity, straight line velocity, lateral head deviation amplitude, linearity, sway, beat cross frequency and straightness. TCS was detected in 74.6% of the samples with a mean of 1.7 µg/L. None of the parameters studied were associated with TCS exposure, although a higher TCS exposure showed higher motility parameters indicating a potential non-linear influence of TCS on sperm quality [74].

In summary, the effects caused by TCS in humans are very similar to those observed in animal models. Spontaneous abortion caused by chronic exposure to TCS is the most observed consequence, although the specific pathway by which it occurs is not yet known. Furthermore, sperm motility is also disrupted by TCS exposure. Thus, we can conclude that fecundity/fertility ends up being the most affected by exposure to TCS due to its influence on both sperm and eggs, which may be a cause of the increased infertility present in developed countries.

5. Effects on the Cardiovascular System

5.1. Animals

Regarding the cardiovascular system, the effect of TCS has not yet been studied as much as the reproductive system; however, some effects on the heart rate and cardiovascular morphology are already known.

In a study conducted by Saley et al., zebrafish were exposed to 1mL of 0, 0.4, 40 or 400 µg/L doses, between 8 and 120 h post fertilisation (hpf), and the dose was changed every 24 h. Doses below 40 µg/L showed minimal changes while a 400 µg/L dose resulted in a dilated auricle, smaller ventricles and unlooping of the heart. The same concentrations were used to assess the cardiac function and structure. The heart rate was reduced by 30% and the beat volume by 40%, which resulted in an output decrease of 60% at the highest dose. At the highest dose, the diastolic end volume was reduced by 17% whilst the systolic volume was unaffected, with 80% of the embryos showing regurgitation [75]. Thus, the authors showed that TCS induces morphological changes in the heart and changes in heart rhythm, namely, the heart rate.

Once more in zebrafish, Wang et al. studied the influence of TCS and its derivatives, 2,4,6-trichlorocatechol and 2,4-dichlorophenol, in cardiotoxicity. The authors used the three compounds in a mixture named TCS-DT at 0.28, 0.56 and 0.84 mg/L, during 6 to 120 hpf in larvae, and they also used 0.14, 0.28 and 0.56 mg/L between 30 and 90 days after fertilisation in adult zebrafish. At 48 hpf, the heart rates of the larvae were significantly reduced in the 0.56 and 0.84 mg/L treatments and the ejection fraction showed a concentration-dependent decrease. At 120 hpf, the looping distance from the atrium to the ventricle increased significantly with the concentration; thus, the TCS-DT exposure affected the cardiac looping and led to abnormal ventricles influencing the cardiac function. Moreover, the creatinine kinase MB and triglyceride levels in the adult hearts increased at all concentrations, the SOD levels increased at 0.14 and 0.28 mg/L, but decreased at 0.56 mg/L, while the lactate dehydrogenase (LDH) levels increased at 0.14 mg/L and decreased with the remaining concentrations. The authors concluded that changes in these activities led to damage in the cardiomyocytes, suggestive of myocarditis. Overall, this demonstrates that exposure to TCS-DT affects normal cardiovascular growth and development in zebrafish [76].

Ma et al. studied the relationship between a TCS-induced abnormal expression of miR-181a-5p and vascular development. The study began by analysing which miRNAs expressions were affected by TCS exposure, which was miR-181a-5p. Zebrafish embryos were treated with 62.5, 125 and 250 µg/L of TCS between 4 and 120 hpf. At the lowest concentration, there was only a small impact on the vascular development and distribution; however, at the other concentrations, a gradual decrease in the blood flow was noted.

For 250 µg/L of TCS, the blood flow was mostly confined in the head; however, there was no distribution of the blood vessels or blood flow in the forebrain and diencephalon. The distribution of red blood cells decreased by 60% in the group treated with 250 µg/L. Additionally, exposure to TCS led to an increase in the miR-181a-5p expression. When miR-181a-5p inhibitors were injected, the number of blood cells increased, while an injection of an analogue of miR-181a-5p decreased the number of blood cells. Thus, the authors concluded that the increased expression of miR-181a-5p was due to exposure to TCS, causing atrophy of the blood vessel development in zebrafish [77].

Cherednichenko et al. analysed the effect of TCS on ryanodine 1 and 2 receptors in rats. After an administration of 6.25, 12.5, or 25 mg/kg of TCS, the authors observed several cardiovascular deficits in the rats including a reduced cardiac output, reduced diastolic volume in the left ventricle, and a reduced maximum time derived from the left ventricular pressure development. The highest dose group exhibited a 25.3 ± 15.7% decrease of the cardiac output, implying that TCS had severe cardiovascular effects. There was an 18% decrease of the exerted force which was significantly lower than the vehicle and placebo groups after a TCS dose of 40 mg/kg. These deficits were reversible as the rats regained full strength 24 h after the TCS administration [78].

More recently, the effect of TCS on vascular contractility was evaluated using the aorta and mesenteric arteries of Sprague-Dawley rats. In mesenteric arteries contracted with phenylephrine, triclosan induced a concentration-dependent relaxation for arteries with and without endothelium. Similarly, when vasoconstriction was induced by high concentrations of potassium, a treatment with triclosan induced relaxation in the mesenteric arteries with and without endothelium. It was concluded that the mesenteric artery relaxation induced by the triclosan was independent of the endothelium. Moreover, when examining the arteries incubated with triclosan 20 min before a vasoconstriction with phenylephrine and KPSS (a high potassium physiological solution), the authors determined that triclosan significantly inhibited the contraction. In the aorta, similar results were observed as in the mesenteric arteries; however, a higher concentration of triclosan was needed for the relaxation to occur [79].

In summary, it can be stated that TCS affects the cardiovascular system in fish and rats. The effects range from vascular relaxation to an alteration of the cardiac morphology and heart rate. Furthermore, these studies also show that not all changes caused by TCS are irreversible, as presented by Cherednichenko et al., as 24 h after TCS administration, the rats recovered from some of the deficits presented. However, the molecular mechanisms underlying these cardiovascular changes remain unclear.

5.2. Humans

Regarding the effects of TCS in humans at the cardiovascular level, the literature is still very scarce, with only two epidemiological studies and two in vitro studies with human cells.

The first study to demonstrate this association between cardiovascular diseases and TCS was performed by Cullinan et al. [17]. In this study, the use of toothpaste with TCS (0.3% *m*/*m*) was analysed over a 5 year period in 438 patients aged up to 75 years with cardiovascular diseases (admitted to the hospital for unstable angina, abnormal electrocardiogram, and myocardial infarction). The authors observed that the prolonged use of toothpaste did not lead to an increase in adverse events or secondary cardiovascular events. Only a small decrease in time to the first serious adverse event was noted between men in the triclosan group and the placebo group [17].

Resorting to the same population sample, the same research group demonstrated the effects of using toothpaste with TCS (0.3% *m*/*m*) for 5 years on the inflammatory biomarkers of cardiovascular diseases. These biomarkers included the total cholesterol, C-reactive protein, low density lipoprotein and endothelial dysfunction. The authors observed no differences between the placebo group and the TCS group, with only slight decreases or increases in the serum levels of each of the biomarkers, except for low density lipoprotein, which decreased in the TCS group compared to the placebo group. Thus, the authors

concluded that the use of toothpaste with 0.3% TCS has a minimal influence on systemic markers in patients with cardiovascular disease, showing no clinical significance in terms of reducing the risk of cardiovascular disease [80].

Regarding the in vitro studies, human embryonic stem cells (hESC) were used, as they possess the ability to differentiate into cardiomyocytes (CMs). In this study, exposure to TCS (1 μM) inhibited the differentiation of hESCs into cardiomyocytes and their spontaneous beating. This inhibition occurred through the interference of triclosan with the gene expression (of ACTC1, GATA4, and TNNT2) and with excessive methylations in the DNA [81].

A study conducted by Zhang et al., resorting to the use of HUVECs (human umbilical cord vein endothelial cells), was also performed. In the first group, the cells were treated with several doses of TCS for 24 h (0, 10, 20, 50 and 100 μM), and in the second group the cells were first treated with SC79 (PI3K/Akt activator) or MHY1485 (mTOR activator) and then treated with TCS at 50 μM for 24 h. After treatment with the activators and TCS for 24 h, the authors demonstrated that these activators were not affected by the dysfunction caused in HUVECs. In order to verify if TCS induced apoptosis in the HUVECs, a LDH test was performed demonstrating an increase in the membrane permeability, and in the caspases 3/7 activity. The levels of BAX and Bcl-2 were also calculated, where the Bcl-2 and BAX levels decreased and increased, respectively. These data demonstrate that TCS does indeed promote endothelial cell apoptosis. Then, the authors also analysed the mechanism by which TCS induced reactive oxygen species production in HUVECs and the results showed that the phosphorylation of PI3k, Akt, mTORC1 and mTORC2 was suppressed. The final test analysed the migration ability of HUVECs after exposure to TCS by wounding the culture at confluence, showing that TCS significantly reduced the regeneration ability compared to the control [82].

In short, TCS at the maximum concentrations currently allowed (e.g., 0.3% (*m*/*m*)) does not appear to have significant effects when ingested; however, in in vitro studies that have already been conducted, this compound at much higher concentrations, was found to affect the cell viability, to induce apoptosis and to decrease considerably the capacity for cell regeneration. Thus, we can conclude that studies in humans are still too scarce to infer an effect of this compound at the cardiovascular level, despite epidemiological studies pointing in that direction; therefore, it is of paramount importance to see if this compound bioaccumulates and if the doses allowed by law are really safe for human health.

6. Effects on the Thyroid

6.1. Animals

Regarding studies on thyroid function, these are still very scarce, although the first study was conducted 15 years ago.

In 2007, Crofton et al. exposed female rats (Long Evans) aged 21–23 days to 0, 10, 30, 100, 300 and 1000 mg/kg/day of TCS for 4 days to test the hypothesis that exposure to TCS altered T4 (thyroxine) levels. The authors found that the serum T4 concentration decreased by 28%, 34% and 53% in the 100, 300 and 1000 mg/kg/day triclosan treatments, respectively. The study demonstrated that triclosan decreased the circulating T4 concentration in female rats [83].

In another study of triclosan toxicity was tested in two different ways. First, Wistar female rats were exposed to TCS (at 75, 150 or 300 mg/kg/day) from GD7 to PND16, and second, the offspring of unexposed females were exposed to TCS (at 0, 50 or 150 mg/kg/day) from PND 3. The T4 levels were analysed in all four groups (e.g., the control, 75, 150 and 300 mg/kg/day) at gestational day 15 (8 days after the beginning of exposure), showing a decrease by 59%, 72% and 72%, respectively, for the three concentrations of TCS, and again at postnatal day 16 (the last day of exposure), where the levels had decreased by 38%, 55% and 58%, respectively, in the females, while in the offspring no significant changes were observed. The authors concluded that TCS can decrease maternal T4 levels during gestation and lactation [11].

Resorting to male Wistar rats, Taha et al. tested the effects of compounds with an oestrogen-like structure, such as TCS, on the thyroid function. The animals were treated with two concentrations of TCS, namely, 10 and 50 mg/kg, for 60 days. The levels of the thyroid hormones, FT3 (free-triiodothyronine), FT4 (free-thyroxine), TSH (thyroid stimulating hormone), T3 (triiodothyronine) and T4, were quantified by the ELISA method. The concentration of 10 mg/kg showed no significant effect for any of the thyroid hormones; however, the concentration of 50 mg/kg led to symptoms of hypothyroidism due to changes in the T3, T4 and TSH levels [84].

Zhang et al. used Sprague-Dawley rats to better understand the mechanism by which triclosan interfered with thyroid homeostasis. After 31 days of treatment with TCS, thyroid hormones were quantified in plasma, and the results showed a decrease in all hormones inversely proportional to the concentration of the TCS administered. At the highest concentration (200 mg/kg/d), TT4 (total thyroxine), FT4, TT3 (total triiodothyronine) and FT3 were decreased by 23.1%, 20.5%, 22.0% and 19.1%, respectively. Only the TSH and TRH (thyrotropin releasing hormone) levels were unaffected. The authors also observed histological changes in the thyroid, dependent of the concentration of TCS used. Subsequently, they analysed by which pathway the TCS affected the thyroid and concluded that besides activating the JNK (c-Jun N-terminal kinase) and p38 pathways in vivo and inducing the p38 pathway from ROS in vitro, there was also an induction of the TRHr (thyrotropin releasing hormone receptor) expression through the p38 pathway, which contributed to the decrease in TPO (thyroid peroxidase). Thus, with this study it was concluded that TCS affected the thyroid homeostasis and histology through the p38/TRHr pathway mediated by TPO [85].

In a different approach, Schnitzler et al. tested whether TCS (20, 50 and 100 µg/L) affected the ontogenetic variation of thyroid hormones in developing *Cyprinodon variegatus* larvae. The total thyroid hormone concentrations were measured at 9, 12, 15 and 18 days post-hatching (dph). The results showed that the TT4 levels varied significantly in all conditions while the control had a gradual decrease. The T4 concentrations increased up to 15 dph, which was not observed for the T3 levels or the T3:T4 ratio. The peak of TT3 observed at the 20 µg/L of TCS concentration occurred 2 days after the control, being indicative of the metamorphosis climax in *Cyprinodon variegatus* [86].

In summary, TCS decreases the serum levels of T4, T3 and TSH in rats, affecting the thyroid homeostasis and histology and leading to symptoms of hypothyroidism. The same was not found in larvae where the T4 levels increased and there were no significant changes in the other hormones; thus, further studies are needed in this area as the effects of TCS on the thyroid function in animals are not yet fully defined.

6.2. Humans

As for the TCS effects on human thyroid function, the few studies performed so far seem to show no adverse effects.

In 2012, the use of toothpastes containing triclosan (0.3% *m*/*m*) for 4 years was analysed and the thyroid effects were observed. All 132 patients, aged up to 75 years old, had a history of hospital admission for myocardial infarction, unstable angina, and abnormal electrocardiogram. The authors measured the TSH, FT4, FT3, anti-TGb and anti-thyroid peroxidase antibody levels; however, no significant association was observed [87].

From the Health Outcomes and Measures of the Environment (HOME) study 2003–2006, Braun et al. analysed the relationship between maternal (202 women), neonatal (274 infants) and childhood (153 children) urinary TCS concentration and the corresponding serum thyroid hormone levels. The concentration of TCS in urine at 16 weeks of gestation decreased slightly until birth; however, the opposite occurred in children aged between 1–3 years. At 16 weeks' gestation, there was no significant association between the TCS levels and thyroid hormone concentration. At the time of delivery, there was an inverse relationship between TCS and the TT4 and FT3 levels; however, due to the size of the population that was used

during the study, the authors could not state that an exposure to TCS would affect the TT4 and FT3 levels [88].

Koeppe et al., using NHANES (National Health and Nutrition Examination Survey) data from 2007–2008, counted the serum levels of both free and total T3 and T4, thyroglobulin and TSH in 352 adolescents (aged 12–19 years) and 1479 adults (aged 20 years and older), and compared them with TCS concentrations measured in the urine samples collected. A positive and significant relationship between TCS and the T3 concentrations was observed only in the adolescents [89].

Berger et al. analysed the relationship between the presence of different chemicals, including TCS, with the serum levels of thyroid hormones in 338 pregnant women (aged 18–45 years) and 364 new-borns. The levels of TT4, FT4 and TSH were measured in the pregnant women while in the new-borns, only the TSH was counted. The results showed an inverse relationship between the urinary TCS concentration and the serum TT4 levels; however, no relationship was found between the urinary TCS concentrations and the TSH levels in the new-borns. It was, therefore, concluded that exposure to TCS may influence thyroid hormone levels during pregnancy [90].

More recently in 2019, the TSH, FT4, TT4, FT3, TT3, thyroid peroxidase antibody (TPOAb) and thyroglobulin antibody (TgAb) levels were measured in pregnant women at week 10 of gestation. There was no association between triclosan with the absolute TSH, FT4 or FT3 concentrations. There was no significant association between TCS and thyroid function throughout pregnancy. The association of TCS with the TSH or FT4 also had no changes with a variation of TPOAb. The association of FT4 with the TSH did not change with the various concentrations of TCS [91]. Thus, the authors concluded that TCS did not affect the thyroid function.

In a study conducted by the Korean National Environmental Health Survey (KoNEHS), the relationship between TCS exposure and thyroid hormones (namely, TT3, TT4 and TSH) was tested. The study population consisted of 2630 men and 3360 women, who provided blood and urine samples. There was a positive association between TCS exposure and the serum TSH levels in the women; however, in the men, no significant changes were observed. As for the serum levels of TT3 and TT4, no changes were noted in either the women or men. These results lead to postulating that TCS may interfere with thyroid function; however, more studies are needed to complement the limitations of this study [92].

In a similar study, using data from the environment and reproductive (EARTH) study, Skarha et al. analysed the interaction between urinary TCS levels with the serum levels of thyroid function biomarkers (namely, TSH, T4, T3, TPOAb and TgAb) in 317 women. They reached the same conclusions as Koeppe et al., with the observed urinary TCS concentrations having had no significant association with the biomarkers of the thyroid function [93].

In summary, most of the studies reviewed did not observe any changes in the thyroid function upon exposure to TCS, with only one study showing an inverse association between the TCS concentration and T4 levels; thus, considering that the lack of associations may be related to a low daily exposure to TCS, more studies are needed to determine the effects of TCS, since the studies performed so far are contradictory.

7. Conclusions and Future Perspectives

From all the studies presented in this review, we can conclude that in animals, TCS has clear effects; however, the same is not verified in humans, which may be due to a greater complexity, a lower TCS exposure or simply due to the difficulty in conducting in vivo studies.

Regarding the effects on the reproductive system, TCS similarly affects both animals and humans. In animals, TCS causes morphological changes in the male rat sexual organs, an increased fertilization time in fish and gestation time in rats and causes spontaneous abortions in sheep by altering the oestrogen sulphotransferase activity. While in humans, of the most common effects, alterations of the sex hormone levels stand out, and also the incidence of spontaneous abortion due to the alteration of oestrogen sulphotransferase

levels as described in animals. It is also possible to observe that TCS affects both the ovarian reserve and sperm morphology and motility.

In the cardiovascular studies, as far as animals were concerned, TCS caused cardiac deficits in fish and mice; however, these were reversible in mice after 24 h. It also induced the relaxation of rat arteries and a long exposure to TCS inhibited their contraction. In humans, more specific conclusions were only drawn in vitro, in which TCS inhibited the differentiation of hESCs and had the ability to induce apoptosis in HUVECs.

Finally, from the studies analysing the thyroid function, various concentrations of TCS were tested in animals, leading to the conclusion that TCS decreased the circulating levels of T4, T3 and TSH and altered the morphology of the thyroid gland; however, in humans, the effects were inconclusive, since the studies analysed had a very small population sample with contradictory results, which reveals the need for further studies relating the TCS effects for the thyroid gland.

Thus, we can conclude that triclosan continues to be poorly studied, especially at the cardiovascular and thyroid levels. In this sense, it is essential to carry out more studies in vitro, ex vivo and in vivo on the effects of TCS on reproduction, especially on male and female fertility, on cardiovascular effects and on the relationship of thyroid hormone levels with physiological concentrations of TCS. Even at a low percentage, humans are still exposed to this endocrine disruptor on a daily basis; therefore, more epidemiological studies with larger population samples, and in different age groups and different countries are needed to really understand what effects TCS has on human health.

In summary, we will only know the true danger, or lack thereof, after this endocrine disruptor is further studied, especially in humans, as it is still a highly used chemical in cosmetic products and disinfectants in the European Union.

Author Contributions: Conceptualization, E.C.; writing—original draft preparation, A.C.M., M.M. and E.C.; writing—review and editing, A.C.M., M.M. and E.C.; supervision, M.M. and E.C.; funding acquisition, E.C. All authors have read and agreed to the published version of the manuscript.

Funding: This work was developed within the scope of the CICS-UBI projects UIDB/00709/2020 and UIDP/00709/2020, financed by national funds through the Portuguese Foundation for Science and Technology/MCTES. Melissa Mariana acknowledges the PhD fellowship from FCT (Reference: 2020.07020.BD).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Bedoux, G.; Roig, B.; Thomas, O.; Dupont, V.; Le Bot, B. Occurrence and toxicity of antimicrobial triclosan and by-products in the environment. *Environ. Sci. Pollut. Res.* **2012**, *19*, 1044–1065. [CrossRef]
2. Yueh, M.F.; Tukey, R.H. Triclosan: A Widespread Environmental Toxicant with Many Biological Effects. *Annu. Rev. Pharmacol. Toxicol.* **2016**, *56*, 251–272. [CrossRef]
3. Weatherly, L.M.; Gosse, J.A. Triclosan exposure, transformation, and human health effects. *J. Toxicol. Environ. Health Part B Crit. Rev.* **2017**, *20*, 447–469. [CrossRef]
4. Shrestha, P.; Zhang, Y.M.; Chen, W.J.; Wong, T.Y. Triclosan: Antimicrobial mechanisms, antibiotics interactions, clinical applications, and human health. *J. Environ. Sci. Health Part C Toxicol. Carcinog.* **2020**, *38*, 245–268. [CrossRef]
5. Tamura, I.; Saito, M.; Nishimura, Y.; Satoh, M.; Yamamoto, H.; Oyama, Y. Elevation of Intracellular Ca^{2+} Level by Triclosan in Rat Thymic Lymphocytes: Increase in Membrane Ca^{2+} Permeability and Induction of Intracellular Ca^{2+} Release. *J. Health Sci.* **2011**, *57*, 540–546. [CrossRef]
6. EC. Commission Regulation (EU) No 358/2014 Amending Annexes II and V to Regulation (EC) No 1223/2009 of the European Parliament and of the Council on Cosmetic Products. 2014. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32014R0358 (accessed on 31 May 2022).
7. Pombo, M.; Castro-Feijoo, L. Endocrine disruptors. *J. Pediatric. Endocrinol. Metab.* **2005**, *18*, 1145–1155. [CrossRef]

8. Benvenga, S.; Elia, G.; Ragusa, F.; Paparo, S.R.; Sturniolo, M.M.; Ferrari, S.M.; Antonelli, A.; Fallahi, P. Endocrine disruptors and thyroid autoimmunity. *Best Pract. Res. Clin. Endocrinol. Metab.* **2020**, *34*, 11. [CrossRef]
9. Monneret, C. What is an endocrine disruptor? *Comptes Rendus Biol.* **2017**, *340*, 403–405. [CrossRef]
10. Diamanti-Kandarakis, E.; Bourguignon, J.P.; Giudice, L.C.; Hauser, R.; Prins, G.S.; Soto, A.M.; Zoeller, R.T.; Gore, A.C. Endocrine-Disrupting Chemicals: An Endocrine Society Scientific Statement. *Endocr. Rev.* **2009**, *30*, 293–342. [CrossRef]
11. Axelstad, M.; Boberg, J.; Vinggaard, A.M.; Christiansen, S.; Hass, U. Triclosan exposure reduces thyroxine levels in pregnant and lactating rat dams and in directly exposed offspring. *Food Chem. Toxicol.* **2013**, *59*, 534–540. [CrossRef]
12. Barbolt, T.A. Chemistry and Safety of Triclosan, and Its Use as an Antimicrobial Coating on Coated VICRYL* Plus Antibacterial Suture (Coated Polyglactin 910 Suture with Triclosan). *Surg. Infect.* **2002**, *3*, s45–s53. [CrossRef]
13. Dann, A.B.; Hontela, A. Triclosan: Environmental exposure, toxicity and mechanisms of action. *J. Appl. Toxicol.* **2011**, *31*, 285–311. [CrossRef]
14. Infarmed. Análise Laboratorial de Produtos de Higiene Oral. 2017. Available online: https://www.infarmed.pt/documents/15786/2215138/Produtos+de+Higiene+Oral/56215a29-68cb-48d5-b32f-650ecbcf0196 (accessed on 31 May 2022).
15. FDA. *Safety and Effectiveness of Consumer Antiseptic Rubs; Topical Antimicrobial Drug Products for over-the-Counter Human Use*; Food and Drug Administration: Silver Spring, MD, USA, 2019; Volume 84.
16. ECHA. Decision on Substance Evaluation Pursuant to Article 46(1) of Regulation (EC) No 1907/2006. 2014. Available online: https://echa.europa.eu/documents/10162/d2148173-ecbf-3f1b-988d-89ba9d06680d (accessed on 31 May 2022).
17. Cullinan, M.P.; Palmer, J.E.; Carle, A.D.; West, M.J.; Westerman, B.; Seymour, G.J. The influence of a triclosan toothpaste on adverse events in patients with cardiovascular disease over 5-years. *Sci. Total Environ.* **2015**, *508*, 546–552. [CrossRef]
18. Szychowski, K.A.; Wnuk, A.; Kajta, M.; Wójtowicz, A.K. Triclosan activates aryl hydrocarbon receptor (AhR)-dependent apoptosis and affects Cyp1a1 and Cyp1b1 expression in mouse neocortical neurons. *Environ. Res.* **2016**, *151*, 106–114. [CrossRef]
19. Szychowski, K.A.; Skóra, B.; Wójtowicz, A.K. Triclosan affects the expression of nitric oxide synthases (NOSs), peroxisome proliferator-activated receptor gamma (PPARγ), and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) in mouse neocortical neurons in vitro. *Toxicol. In Vitro* **2021**, *73*, 105143. [CrossRef]
20. Li, J.; Quan, X.; Zhang, Y.; Yu, T.; Lei, S.; Huang, Z.; Wang, Q.; Song, W.; Yang, X.; Xu, P. PPARγ Regulates Triclosan Induced Placental Dysfunction. *Cells* **2021**, *11*, 86. [CrossRef]
21. Ahn, K.C.; Zhao, B.; Chen, J.; Cherednichenko, G.; Sanmarti, E.; Denison, M.S.; Lasley, B.; Pessah, I.N.; Kultz, D.; Chang, D.P.Y.; et al. In vitro biologic activities of the antimicrobials triclocarban, its analogs, and triclosan in bioassay screens: Receptor-based bioassay screens. *Environ. Health Perspect.* **2008**, *116*, 1203–1210. [CrossRef]
22. Ohtake, F.; Baba, A.; Fujii-Kuriyama, Y.; Kato, S. Intrinsic AhR function underlies cross-talk of dioxins with sex hormone signalings. *Biochem. Biophys. Res. Commun.* **2008**, *370*, 541–546. [CrossRef]
23. Bhargava, H.N.; Leonard, P.A. Triclosan: Applications and safety. *Am. J. Infect. Control* **1996**, *24*, 209–218. [CrossRef]
24. Dhillon, G.S.; Kaur, S.; Pulicharla, R.; Brar, S.K.; Cledon, M.; Verma, M.; Surampalli, R.Y. Triclosan: Current Status, Occurrence, Environmental Risks and Bioaccumulation Potential. *Int. J. Environ. Res. Public Health* **2015**, *12*, 5657–5684. [CrossRef]
25. Olaniyan, L.W.B.; Mkwetshana, N.; Okoh, A.I. Triclosan in water, implications for human and environmental health. *Springerplus* **2016**, *5*, 17. [CrossRef] [PubMed]
26. Fang, J.L.; Stingley, R.L.; Beland, F.A.; Harrouk, W.; Lumpkins, D.L.; Howard, P. Occurrence, Efficacy, Metabolism, and Toxicity of Triclosan. *J. Environ. Sci. Health Part C Environ. Carcinog. Ecotoxicol. Rev.* **2010**, *28*, 147–171. [CrossRef] [PubMed]
27. Lee, J.D.; Lee, J.Y.; Kwack, S.J.; Shin, C.Y.; Jang, H.J.; Kim, H.Y.; Kim, M.K.; Seo, D.W.; Lee, B.M.; Kim, K.B. Risk Assessment of Triclosan, a Cosmetic Preservative. *Toxicol. Res.* **2019**, *35*, 137–154. [CrossRef] [PubMed]
28. SCCS. Scientific Advice on the Safety of Triclocarban and Triclosan as Substances with Potential Endocrine Disrupting Properties in Cosmetic Products. 2022. Available online: https://health.ec.europa.eu/publications/safety-triclocarban-and-triclosan-substances-potential-endocrine-disrupting-properties-cosmetic_en (accessed on 31 May 2022).
29. Sigma-Aldrich. *Triclosan Product Specification*; Sigma-Aldrich: St. Louis, MO, USA, 2022; Available online: https://www.sigmaaldrich.com/PT/en/sds/sial/phr1338 (accessed on 10 January 2022).
30. Belosludtsev, K.N.; Belosludtseva, N.V.; Tenkov, K.S.; Penkov, N.V.; Agafonov, A.V.; Pavlik, L.L.; Yashin, V.A.; Samartsev, V.N.; Dubinin, M.V. Study of the mechanism of permeabilization of lecithin lipossomes and rat liver mitochondria by the antimicrobial drug triclosan. *BBA Biomembr.* **2018**, *1860*, 264–271. [CrossRef]
31. Lygre, H.; Moe, G.; Skalevik, R.; Holmsen, H. Interaction of triclosan with eukaryotic membrane lipids. *Eur. J. Oral Sci.* **2003**, *111*, 216–222. [CrossRef]
32. Wu, J.L.; Liu, J.; Cai, Z.W. Determination of triclosan metabolites by using in-source fragmentation from high-performance liquid chromatography/negative atmospheric pressure chemical ionization ion trap mass spectrometry. *Rapid Commun. Mass Spectrom.* **2010**, *24*, 1828–1834. [CrossRef]
33. Adolfsson-Erici, M.; Pettersson, M.; Parkkonen, J.; Sturve, J. Triclosan, a commonly used bactericide found in human milk and in the aquatic environment in Sweden. *Chemosphere* **2002**, *46*, 1485–1489. [CrossRef]
34. Guillén, J.; Bernabeu, A.; Shapiro, S.; José, V. Location and orientation of Triclosan in phospholipid model membranes. *Eur. Biophys. J.* **2004**, *33*, 448–453. [CrossRef]
35. Allmyr, M.; Adolfsson-Erici, M.; McLachlan, M.S.; Sandborgh-Englund, G. Triclosan in plasma and milk from Swedish nursing mothers and their exposure via personal care products. *Sci. Total Environ.* **2006**, *372*, 87–93. [CrossRef]

36. Lin, Y.J. Buccal absortin of triclosan following topical mouthrinse application. *Am. J. Dent.* **2000**, *13*, 215–217.
37. Aiello, A.E.; Larson, E.L.; Levy, S.B. Consumer Antibacterial Soaps: Effective or Just Risky? *Clin. Infect. Dis.* **2007**, *45*, S137–S147. [CrossRef] [PubMed]
38. Queckenberg, C.; Meins, J.; Wachall, B.; Doroshyenko, O.; Tomalik-Scharte, D.; Bastian, B.; Abdel-Tawab, M.; Fuhr, U. Absorption, Pharmacokinetics, and Safety of Triclosan after Dermal Administration. *Antimicrob. Agents Chemother.* **2010**, *54*, 570–572. [CrossRef] [PubMed]
39. Kanetoshi, A.; Ogawa, H.; Katsura, E.; Okui, T.; Kaneshima, H. Disposition and excretion of irgasan dp300 and its chlorinated derivatives in mice. *Arch. Environ. Contam. Toxicol.* **1988**, *17*, 637–644. [CrossRef]
40. Moss, T.; Howes, D.; Williams, F.M. Percutaneous penetration and dermal metabolism of triclosan (2,4,4′-trichloro-2′-hydroxydiphenyl ether). *Food Chem. Toxicol.* **2000**, *38*, 361–370. [CrossRef]
41. Fang, J.L.; Vanlandingham, M.; da Costa, G.G.; Beland, F.A. Absorption and metabolism of triclosan after application to the skin of B6C3F1 mice. *Environ. Toxicol.* **2016**, *31*, 609–623. [CrossRef]
42. Geens, T.; Neels, H.; Covaci, A. Distribution of bisphenol-A, triclosan and n-nonylphenol in human adipose tissue, liver and brain. *Chemosphere* **2012**, *87*, 796–802. [CrossRef]
43. Wu, Y.F.; Chitranshi, P.; Loukotkova, L.; da Costa, G.G.; Beland, F.A.; Zhang, J.; Fang, J.L. Cytochrome P450-mediated metabolism of triclosan attenuates its cytotoxicity in hepatic cells. *Arch. Toxicol.* **2017**, *91*, 2405–2423. [CrossRef]
44. Zhu, L.Y.; Shao, Y.; Xiao, H.X.; Santiago-Schubel, B.; Meyer-Alert, H.; Schiwy, S.; Yin, D.Q.; Hollert, H.; Kuppers, S. Electrochemical simulation of triclosan metabolism and toxicological evaluation. *Sci. Total Environ.* **2018**, *622*, 1193–1201. [CrossRef]
45. Rodricks, J.V.; Swenberg, J.A.; Borzelleca, J.F.; Maronpot, R.R.; Shipp, A.M. Triclosan: A critical review of the experimental data and development of margins of safety for consumer products. *Crit. Rev. Toxicol.* **2010**, *40*, 422–484. [CrossRef]
46. Wang, L.Q.; Falany, C.N.; James, M.O. Triclosan as a substrate and inhibitor of 3′-phosphoadenosine-5′-phosphosulfate-sulfotransferase and UDP-glucuronosyl transferase in human liver fractions. *Drug Metab. Dispos.* **2004**, *32*, 1162–1169. [CrossRef]
47. Provencher, G.; Berube, R.; Dumas, P.; Bienvenu, J.F.; Gaudreau, E.; Belanger, P.; Ayotte, P. Determination of bisphenol A, triclosan and their metabolites in human urine using isotope-dilution liquid chromatography-tandem mass spectrometry. *J. Chromatogr. A* **2014**, *1348*, 97–104. [CrossRef] [PubMed]
48. Sandborgh-Englund, G.; Adolfsson-Erici, M.; Odham, G.; Ekstrand, J. Pharmacokinetics of triclosan following oral ingestion in humans. *J. Toxicol. Environ. Health Part A Curr. Issues* **2006**, *69*, 1861–1873. [CrossRef] [PubMed]
49. Lorigo, M.; Cairrao, E. Fetoplacental vasculature as a model to study human cardiovascular endocrine disruption. *Mol. Asp. Med.* **2022**, *87*, 38. [CrossRef] [PubMed]
50. Feiteiro, J.; Mariana, M.; Cairrão, E. Health toxicity of brominated flame retardants: From environmental to human exposure. *Environ. Pollut.* **2021**, *285*, 117475. [CrossRef] [PubMed]
51. Mesquita, I.; Lorigo, M.; Cairrao, E. Update about the disrupting-effects of phthalates on the human reproductive system. *Mol. Reprod. Dev.* **2021**, *88*, 650–672. [CrossRef]
52. Azzouz, A.; Rascon, A.J.; Ballesteros, E. Simultaneous determination of parabens, alkylphenols, phenylphenols, bisphenol A and triclosan in human urine, blood and breast milk by continuous solid-phase extraction and gas chromatography-mass spectrometry. *J. Pharm. Biomed. Anal.* **2016**, *119*, 16–26. [CrossRef]
53. Allmyr, M.; Harden, F.; Toms, L.M.L.; Mueller, J.F.; McLachlan, M.S.; Adolfsson-Erici, M.; Sandborgh-Englund, G. The influence of age and gender on triclosan concentrations in Australian human blood serum. *Sci. Total Environ.* **2008**, *393*, 162–167. [CrossRef]
54. Calafat, A.M.; Ye, X.; Wong, L.Y.; Reidy, J.A.; Needham, L.L. Urinary concentrations of triclosan in the U.S. population: 2003-2004. *Env. Health Perspect.* **2008**, *116*, 303–307. [CrossRef]
55. Jin, C.; Chen, Y.; Zhang, P.; Xiong, Z.; Wang, C.; Tian, Y. Advances on research of human exposure to triclosan. *Chin. J. Prev. Med.* **2016**, *50*, 285–288. [CrossRef]
56. Macisaac, J.K.; Gerona, R.R.; Blanc, P.D.; Apatira, L.; Friesen, M.W.; Coppolino, M.; Janssen, S. Health Care Worker Exposures to the Antibacterial Agent Triclosan. *J. Occup. Environ. Med.* **2014**, *56*, 834–839. [CrossRef]
57. Ishibashi, H.; Matsumura, N.; Hirano, M.; Matsuoka, M.; Shiratsuchi, H.; Ishibashi, Y.; Takao, Y.; Arizono, K. Effects of triclosan on the early life stages and reproduction of medaka Oryzias latipes and induction of hepatic vitellogenin. *Aquat. Toxicol.* **2004**, *67*, 167–179. [CrossRef] [PubMed]
58. Liu, M.; Ai, W.M.; Sun, L.M.; Fang, F.; Wang, X.D.; Chen, S.B.; Wang, H.L. Triclosan-induced liver injury in zebrafish (Danio rerio) via regulating MAPK/p53 signaling pathway. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* **2019**, *222*, 108–117. [CrossRef] [PubMed]
59. Yingjie, Q.; Jiayi, H.; Ping, H.; Jiangbo, Q.; Xubo, W.; Jun, W. Long-term exposure to environmental relevant triclosan induces reproductive toxicity on adult zebrafish and its potential mechanism. *Sci. Total Environ.* **2022**, *826*, 154026.
60. Kumar, V.; Chakraborty, A.; Kural, M.R.; Roy, P. Alteration of testicular steroidogenesis and histopathology of reproductive system in male rats treated with triclosan. *Reprod. Toxicol.* **2009**, *27*, 177–185. [CrossRef]
61. Stoker, T.E.; Gibson, E.K.; Zorrilla, L.M. Triclosan Exposure Modulates Estrogen-Dependent Responses in the Female Wistar Rat. *Toxicol. Sci.* **2010**, *117*, 45–53. [CrossRef]
62. Jung, E.M.; An, B.S.; Choi, K.C.; Jeung, E.B. Potential estrogenic activity of triclosan in the uterus of immature rats and rat pituitary GH3 cells. *Toxicol. Lett.* **2012**, *208*, 142–148. [CrossRef]

63. Louis, G.W.; Hallinger, D.R.; Stoker, T.E. The effect of triclosan on the uterotrophic response to extended doses of ethinyl estradiol in the weanling rat. *Reprod. Toxicol.* **2013**, *36*, 71–77. [CrossRef]
64. Crawford, B.R.; deCatanzaro, D. Disruption of blastocyst implantation by triclosan in mice: Impacts of repeated and acute doses and combination with bisphenol-A. *Reprod. Toxicol.* **2012**, *34*, 607–613. [CrossRef]
65. Wang, X.L.; Chen, X.J.; Feng, X.J.; Chang, F.; Chen, M.J.; Xia, Y.K.; Chen, L. Triclosan causes spontaneous abortion accompanied by decline of estrogen sulfotransferase activity in humans and mice. *Sci. Rep.* **2015**, *5*, 11. [CrossRef]
66. Feng, Y.X.; Zhang, P.; Zhang, Z.B.; Shi, J.C.; Jiao, Z.H.; Shao, B. Endocrine Disrupting Effects of Triclosan on the Placenta in Pregnant Rats. *PLoS ONE* **2016**, *11*, 14. [CrossRef]
67. Montagnini, B.G.; Forcato, S.; Pernoncine, K.V.; Monteiro, M.C.; Pereira, M.R.F.; Costa, N.O.; Moreira, E.G.; Anselmo-Franci, J.A.; Gerardin, D.C.C. Developmental and Reproductive Outcomes in Male Rats Exposed to Triclosan: Two-Generation Study. *Front. Endocrinol.* **2021**, *12*, 738980. [CrossRef] [PubMed]
68. Raj, S.; Sen Singh, S.; Singh, S.P.; Singh, P. Evaluation of Triclosan-induced reproductive impairments in the accessory reproductive organs and sperm indices in the mice. *Acta Histochem.* **2021**, *123*, 9. [CrossRef] [PubMed]
69. James, M.O.; Li, W.J.; Summerlot, D.P.; Rowland-Faux, L.; Wood, C.E. Triclosan is a potent inhibitor of estradiol and estrone sulfonation in sheep placenta. *Environ. Int.* **2010**, *36*, 942–949. [CrossRef]
70. Wang, C.F.; Tian, Y. Reproductive endocrine-disrupting effects of triclosan: Population exposure, present evidence and potential mechanisms. *Env. Pollut.* **2015**, *206*, 195–201. [CrossRef]
71. Zhang, N.; Wang, W.S.; Li, W.J.; Liu, C.; Chen, Y.Y.; Yang, Q.L.; Wang, Y.; Sun, K. Inhibition of 11 beta-HSD2 Expression by Triclosan via Induction of Apoptosis in Human Placental Syncytiotrophoblasts. *J. Clin. Endocrinol. Metab.* **2015**, *100*, E542–E549. [CrossRef] [PubMed]
72. Etzel, T.M.; Calafat, A.M.; Ye, X.Y.; Chen, A.M.; Lanphear, B.P.; Savitz, D.A.; Yolton, K.; Braun, J.M. Urinary triclosan concentrations during pregnancy and birth outcomes. *Environ. Res.* **2017**, *156*, 505–511. [CrossRef]
73. Jurewicz, J.; Wielgomas, B.; Radwan, M.; Karwacka, A.; Klimowska, A.; Dziewirska, E.; Korczak, K.; Zajdel, R.; Radwan, P.; Hanke, W. Triclosan exposure and ovarian reserve. *Reprod. Toxicol.* **2019**, *89*, 168–172. [CrossRef]
74. Yuan, G.X.; Ma, Y.; Zeng, Y.X.; Pan, H.B.; Liu, P.Y.; Liu, Y.; Liu, G.H.; Cheng, J.Q.; Guo, Y.S. Associations between low-dose triclosan exposure and semen quality in a Chinese population. *Environ. Pollut.* **2022**, *299*, 7. [CrossRef]
75. Saley, A.; Hess, M.; Miller, K.; Howard, D.; King-Heiden, T.C. Cardiac Toxicity of Triclosan in Developing Zebrafish. *Zebrafish* **2016**, *13*, 399–404. [CrossRef]
76. Wang, D.T.; Zhang, Y.H.; Li, J.Y.; Dahlgren, R.A.; Wang, X.D.; Huang, H.S.; Wang, H.L. Risk assessment of cardiotoxicity to zebrafish (Danio rerio) by environmental exposure to triclosan and its derivatives. *Environ. Pollut.* **2020**, *265*, 12. [CrossRef]
77. Ma, Y.; Zang, L.X.; Wang, D.T.; Jiang, J.H.; Wang, C.H.; Wang, X.D.; Fang, F.; Wang, H.L. Effects of miR-181a-5p abnormal expression on zebrafish (Danio rerio) vascular development following triclosan exposure. *Chemosphere* **2019**, *223*, 523–535. [CrossRef]
78. Cherednichenko, G.; Zhang, R.; Bannister, R.A.; Timofeyev, V.; Li, N.; Fritsch, E.B.; Feng, W.; Barrientos, G.C.; Schebb, N.H.; Hammock, B.D.; et al. Triclosan impairs excitation-contraction coupling and Ca2+ dynamics in striated muscle. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 14158–14163. [CrossRef] [PubMed]
79. Zhang, X.Y.; Zhang, X.Z.; Zhang, Y.Q.; Liu, M.Y.; Jin, J.; Yan, J.; Shen, X.; Hu, N.; Dong, D.L. Mitochondrial uncoupler triclosan induces vasorelaxation of rat arteries. *Acta Pharm. Sin. B* **2017**, *7*, 623–629. [CrossRef] [PubMed]
80. Cullinan, M.P.; Palmer, J.E.; Faddy, M.J.; Westerman, B.; Carle, A.D.; West, M.J.; Seymour, G.J. The Influence of Triclosan on Biomarkers of Cardiovascular Risk in Patients in the Cardiovascular and Periodontal Study (CAPS): A Randomized Controlled Trial. *J. Periodontol.* **2015**, *86*, 847–855. [CrossRef] [PubMed]
81. Du, G.Z.; Yu, M.M.; Wang, L.L.; Hu, W.Y.; Song, L.; Lu, C.C.; Wang, X.R. Transcriptome and DNA Methylome Dynamics during Triclosan- Induced Cardiomyocyte Differentiation Toxicity. *Stem Cells Int.* **2018**, *2018*, 8. [CrossRef] [PubMed]
82. Zhang, M.; Zhu, R.R.; Zhang, L.B. Triclosan stimulates human vascular endothelial cell injury via repression of the PI3K/Akt/mTOR axis. *Chemosphere* **2020**, *241*, 7. [CrossRef]
83. Crofton, K.M.; Paul, K.B.; De Vito, M.J.; Hedge, J.M. Short-term in vivo exposure to the water contaminant triclosan: Evidence for disruption of thyroxine. *Environ. Toxicol. Pharmacol.* **2007**, *24*, 194–197. [CrossRef] [PubMed]
84. Taha, M.; Marie, A.M.; Ahmed-Farid, O.A. Combined approaches for evaluation of xenoestrogen neural toxicity and thyroid dysfunction: Screening of oxido-nitrosative markers, DNA fragmentation, and biogenic amine degradation. *J. Biochem. Mol. Toxicol.* **2020**, *34*, 10. [CrossRef]
85. Zhang, P.; Yang, M.; Zeng, L.; Liu, C.J. P38/TRHr-Dependent Regulation of TPO in Thyroid Cells Contributes to the Hypothyroidism of Triclosan-Treated Rats. *Cell. Physiol. Biochem.* **2018**, *45*, 1303–1315. [CrossRef]
86. Schnitzler, J.G.; Frederich, B.; Dussenne, M.; Klaren, P.H.M.; Silvestre, F.; Das, K. Triclosan exposure results in alterations of thyroid hormone status and retarded early development and metamorphosis in Cyprinodon variegatus. *Aquat. Toxicol.* **2016**, *181*, 1–10. [CrossRef]
87. Cullinan, M.P.; Palmer, J.E.; Carle, A.D.; West, M.J.; Seymour, G.J. Long term use of triclosan toothpaste and thyroid function. *Sci. Total Environ.* **2012**, *416*, 75–79. [CrossRef] [PubMed]
88. Braun, J.M.; Chen, A.M.; Hoofnagle, A.; Papandonatos, G.D.; Jackson-Browne, M.; Hauser, R.; Romano, M.E.; Karagas, M.R.; Yolton, K.; Zoeller, R.T.; et al. Associations of early life urinary triclosan concentrations with maternal, neonatal, and child thyroid hormone levels. *Horm. Behav.* **2018**, *101*, 77–84. [CrossRef] [PubMed]

89. Koeppe, E.S.; Ferguson, K.K.; Colacino, J.A.; Meeker, J.D. Relationship between urinary triclosan and paraben concentrations and serum thyroid measures in NHANES 2007–2008. *Sci. Total Environ.* **2013**, *445*, 299–305. [CrossRef] [PubMed]
90. Berger, K.; Gunier, R.B.; Chevrier, J.; Calafat, A.M.; Ye, X.Y.; Eskenazi, B.; Harley, K.G. Associations of maternal exposure to triclosan, parabens, and other phenols with prenatal maternal and neonatal thyroid hormone levels. *Environ. Res.* **2018**, *165*, 379–386. [CrossRef] [PubMed]
91. Derakhshan, A.; Shu, H.; Peeters, R.P.; Kortenkamp, A.; Lindh, C.H.; Demeneix, B.; Bornehag, C.G.; Korevaar, T.I.M. Association of urinary bisphenols and triclosan with thyroid function during early pregnancy. *Environ. Int.* **2019**, *133*, 7. [CrossRef]
92. Ha, N.Y.; Kim, D.H.; Ryu, J.Y. Relationship between triclosan exposure and thyroid hormones: The Second Korean National Environmental Health Survey (2012–2014). *Ann. Occup. Environ. Med.* **2019**, *31*, 9. [CrossRef]
93. Skarha, J.; Minguez-Alarcon, L.; Williams, P.L.; Korevaar, T.I.M.; de Poortere, R.A.; Broeren, M.A.C.; Ford, J.B.; Eliot, M.; Hauser, R.; Braun, J.M. Cross-sectional associations between urinary triclosan and serum thyroid function biomarker concentrations in women. *Environ. Int.* **2019**, *122*, 256–262. [CrossRef]