Substance Name: Resorcinol

EC Number: 203-585-2

CAS Number: 108-46-3

SUPPORT DOCUMENT TO THE OPINION OF THE MEMBER STATE COMMITTEE ON IDENTIFICATION OF

RESORCINOL

AS A SUBSTANCE OF VERY HIGH CONCERN BECAUSE OF ITS ENDOCRINE DISRUPTING PROPERTIES (ARTICLE 57(F) - HUMAN HEALTH)

Adopted on 12 June 2020

This document has been prepared according to template: TEM-0049.03

CONTENTS

TABLES

FIGURES

List of abbreviations

ADHD: Attention deficit hyperactivity disorder
AntiTG Ab: antithyroglobulin antibody
AntiMS Ab, antimicrosomal antibody
AhR: aryl hydrocarbon receptor
AOP: adverse outcome pathway
AR: androgen receptor
AUC: area under curve
AUR : amplex ultrared
BMD: benchmark dose
BSA: bovine serum albumin
BW: body weight
cAMP: cyclic adenosine monophosphate
CH: congenital hypothyroidism
CICADS: Concise International Chemical Assessment Documents
CoRAP: community rolling action plan
DIT: diiodotyrosine
DNA: deoxyribonucleic acid
DMSO: dimethylsulfoxide
E2:17β-estradiol
EATS: Estrogen, Androgen, Thyroid and Steroidogenesis
EC: European commission
EC50: half effective concentration
ECHA: European Chemicals Agency
ED: endocrine disruptor
EFSA: European Food Safety Agency
ELoC: equivalent level of concern
ENDOMET: project QLRT-02637funded by the EC 5th Framework programme
ER: estrogen receptor
ERE: estrogen response element
F: female
FELS: fish early-life stage (OECD 210)
FOB: functional observation battery
FTI: free thyroxine index
FRTL: thyroid cell line established from normal thyroid glands from 5 to 6-week-old Fischer rats
FSH: follicle stimulating hormone
FT3: free T3
FT4: free T4
GC/MS: gas chromatography/ mass spectrometry
GD: gestation day
GSK: glycogen synthase kinase
GWE: goitrogenic water extract
Hb: hemoglobin
HCD: historical control database
hER: human estrogen receptor
Hpf: hours post-fertilisation
HPT: hypothalamic-pituitary-thyroid
I: iodine
IC50: half inhibitory concentration
IPCS: international programme on chemical safety
IOEL: indicative occupational exposure limit
IQ: intelligence quotient
IT4C: intrafollicular T4 content
IUCLID: International Uniform ChemicaL Information Database
Iv: intravenous
JRC: Joint Research Center
KER: key event relationship
LAGDA : Larval Amphibian Growth and Development Assay
LDL: Low Density Lipoproteins
LEV: local exhaust ventilation
LOD: level of detection
LOEC: Lowest Observed Effect Concentration
LOQ: level of quantification
LPO: lactoperoxidase
M: male
MCT: monocarboxylate transporter
MH: maternal hypothyroidism
MIE: molecular initiating event
MIT: monoiodothyrosine
MMI: methimazole
MoA: mode of action
MOE: margin of exposure
MTU: methylthiouracil
ND: no data
NIS: Na/I symporter
NO(A)EL: no observed (adverse) effect level
OECD: organisation for economic co-operation and development
OEL: occupational exposure limit
PBI: protein bound index

PBPK/PD: physiologocally based pharmacokinetic /pharmacodynamic model
PBS: phosphate buffered saline
PC: product category
PND: postnatal day
PRF: Phenol Resorcinol Formaldehyde
PTU: propylthiouracil
T3: triiodothyronine
T4: thyroxine
T7 or free thyroxine index (FTI): total T4 multiplied by T3 uptake
TCDD: 2,3,7,8-tetrachlorodibenzo-p-dioxine
TDI: thyroid disrupting index
Tg: thyroglobulin
TH: thyroid hormone
TIF2: transcriptional intermediary factor 2
TPO: thyroperoxidase
TSH: thyroid-stimulating hormone
TWA: time weighted average
RBC: red blood cell
RF: Resorcinol Formaldehyde
RNA: ribonucleic acid
ROS: reactive oxygen species
SCCS: Scientific Committee on Consumer Safety
SD: standard deviation
SEv: substance evaluation
SMAC: Sequential Multiple Analysis - Computer
SVHC: substance of very high concern
UNEP: United Nations environment programme
USR: unpublished study report
WHO: World Health Organisation
WoE: weight of evidence

IDENTIFICATION OF A SUBSTANCE OF VERY HIGH CONCERN ON THE BASIS OF THE CRITERIA SET OUT IN REACH ARTICLE 57

Substance Name: resorcinol

EC Number: 203-585-2

CAS number: 108-46-3

- The substance should be identified as a substance of equivalent level of concern to those of other substances listed in points (a) to (e) of Article 57 of Regulation (EC) No 1907/2006 (REACH) according to Article 57(f) of the REACH Regulation.

Summary of how the substance meets the criteria set out in Article 57 of the REACH Regulation

Resorcinol should be identified as a substance of very high concern in accordance with Article 57(f) of Regulation (EC) 1907/2006 (REACH) because it is a substance with endocrine disrupting properties for which there is scientific evidence of probable serious effects to human health which gives rise to an equivalent level of concern to those of other substances listed in points (a) to (e) of Article 57 REACH.

ED properties of resorcinol relevant for human health

Resorcinol fulfils the definition of an endocrine disruptor relevant for human health on the following basis:

It is well established based on a series of case reports, where high doses of resorcinol were given, that severe hypothyroidism[1] was diagnosed and reversed when exposure to resorcinol was stopped. This leads to the conclusion that exposure to resorcinol can affect the regulation of the thyroid function inducing hypothyroidism in humans. The endocrine disrupting properties of resorcinol have therefore been demonstrated in humans under these specific conditions of exposure.

The medical cases reporting these effects are patients that applied resorcinol to damaged skin for 9 out of 10 cases. However, considering that the skin was reported as intact in one additional case and that skin absorption has been demonstrated *in vitro*, these data are considered relevant in the assessment.

In addition, findings consistent with the MoA (mode of action) of thyroid disruption via TPO (thyroperoxidase) inhibition are also reported in several experimental studies via drinking water. Similar findings reported in studies conducted by subcutaneous, dietary and inhalation routes provide supportive evidence. In particular histopathological changes in the thyroid and changes in the circulating levels of T3 (triiodothyronine) or T4 (thyroxine), are considered as adverse effects.

Inhibition of TPO by resorcinol, a key enzyme in the synthesis of thyroid hormones, is established *in vitro* in several experimental designs.

[1] Hypothyroidism is characterised by low FT4 and high TSH

Altogether, the effects observed in humans and in some experimental studies are fully consistent with the MoA via TPO inhibition. Based on current knowledge, the biological plausibility of a causal link between inhibition of TPO, disruption of thyroid hormone levels and adverse effects linked to low thyroid hormone levels is strong. A recently validated AOP (adverse outcome pathway) described the relationship between inhibition of TPO, decreased T4 and neurodevelopmental alteration due to maternal low T4 concentration as having a high level of evidence for humans (AOP n°42[2]). With the validation of this AOP by the OECD, further experimental data investigating (neuro)development are not necessary to confirm the effects observed in the preliminary two-generation study available in the registration dossier.

Based on a weight of evidence analysis of the available data, there is scientific evidence that resorcinol can have adverse effects on human health through thyroid disruption and fulfils the definition of an endocrine disruptor.

Overall assessment of an equivalent level of concern

Due to the ED properties of resorcinol, it is a substance of equivalent level of concern as specified in article 57 of REACH on the following basis:

- Human cases have been identified under specific conditions and this may raise some questions on their relevance for actual conditions of human exposure. The effects induced by resorcinol in humans and in experimental animals are complementary in the demonstration that they cannot be considered as specific to exceptional conditions of exposure, in particular because some populations and periods of exposure can be associated with specific sensitivity. Besides, the possibility that resorcinol may induce effects in humans under common conditions of exposure can not be excluded on a toxico-kinetic basis.

- Hypothyroidism has clinical implications related to nearly all major organs. It is a serious condition and the capacity for resorcinol to induce or contribute to existing (subclinical[3]) hypothyroidism raises significant concern. Alterations of foetal development, in particular brain development as a consequence of low maternal levels in TH, have been associated with serious adverse health outcomes.

- Although generally considered reversible, hypothyroidism raises concern because of the delay in the onset of symptoms. Effects can be compensated for in the short-term but it may result in severe forms that appear after long-term exposure. In addition, less severe forms are expected to be more difficult to identify because symptoms are generally unspecific. This results in delays in diagnosis and treatment. In contrast, the neurodevelopmental effects expected as a consequence of maternal low concentration of T4 have consequences that are observed later in life, without a direct exposure. They are considered as permanent and irreversible and raise an additional concern. The difficulty posed by latency both in terms of detection of the effect and induction of developmental effects is considered as an additional concern.

- The adverse effects induced or enhanced by resorcinol may lead to a reduced quality of life.

- Thyroid-disrupting chemicals may have a role in the increased incidence of pathologies of the thyroid or conditions associated with thyroid dysfunction, in

[2] https://aopwiki.org/aops/42
[3] Subclinical hypothyroidism is characterised by normal FT4 and high TSH

particular hypothyroidism, thyroid cancer and neurodevelopmental disorders. This raises a societal concern.

- A number of uncertainties to derive a 'safe concentration' have emerged considering the resorcinol data as detailed below as well as considering recent developments in the understanding of the consequences of thyroid disruption.
    - The potency of TPO inhibition can be modulated by several factors, in particular iodine levels.
    - Considering the wide range of functions influenced by TH (thyroid hormone), it is also highly challenging to fully characterise these effects and their dose-response in experimental studies. In particular, neurodevelopmental effects are generally considered as the most sensitive. There is some evidence indicating a possible neurodevelopmental effect of resorcinol but that has not been investigated in detail.
    - Lack of information on internal levels of exposure (plasma concentration) in human cases and on incidence of less severe hypothyroidism preclude the estimation of a dose-response relationship in humans.
    - The discrepancy between experimental results depending on the route of administration is not fully understood. In particular, internal exposure and toxicokinetic differences depending on route of exposure and vehicle have not been fully characterised. Comparing the severity of effects between rodents and humans, a higher sensitivity of humans or certain humans cannot be excluded. Depending on the route of administration, some effects have been observed in experimental studies at doses associated with low systemic concentrations of resorcinol in experimental studies. Large uncertainties remain on the level of systemic exposure to resorcinol that induces effects on the thyroid function. Additional uncertainty remains on the level of systemic exposure to resorcinol after different routes of exposure.
    - Hypothyroidism is among the most common of endocrine conditions and resorcinol can aggravate existing conditions. Any additional thyroid disturbance by resorcinol may in some instances counteract part of the compensatory mechanisms developed by the thyroid gland, namely increased expression of TPO, and could aggravate an existing condition that is of relatively common prevalence although often undiagnosed. Pregnancy is also a period that is highly sensitive to disruption of TH regulation because of a higher need for TH and physiological changes affecting their toxicokinetics. Besides, the importance of even modest changes in TH during pregnancy has emerged and strengthened recently. The results of a comprehensive review by the US EPA (2019) lend support to the concept that maternal FT4 (free T4), especially in the hypothyroxinemic range[4], is critical to proper neurodevelopment of the offspring. Across different age ranges and neurodevelopmental indices, the impact of altered FT4 is seen even with small incremental changes in FT4. Pregnancy is therefore likely to be a period of sensitivity to the alteration of TH regulation by resorcinol, with consequences for the neurodevelopment of the offspring that can be affected even by small changes. A number of vulnerable populations may therefore be of particular concern. Sensitive populations include those with undiagnosed subclinical hypothyroidism, marginal dietary iodine deficiency, pregnant women, the developing foetus, the newborn, and young infant – all of which may be particularly susceptible to TH disruption induced by

[4] Hypothyroxinemia is characterised by low FT4 and normal TSH

resorcinol. Establishing safe levels for these particularly sensitive populations is surrounded with large uncertainties due to the lack of agreed parameters to identify such persons and high symptom variability in relation to the physiological status.

Some of the effects that resorcinol may induce in relation to its thyroid-disrupting potential are serious and are irreversible or may not be detectable without delay. They can impact on the quality of life and raise societal concern of a high and increasing burden. Most importantly, the difficulty to establish a safe level with sufficient certainty raises concern on the capacity to manage safe use of the substance in particular for sensitive populations and with the emergence of the understanding that small changes in maternal T4 can affect brain development of the offspring. Altogether, this gives rise to an equivalent level of concern to those of other substances listed in points (a) to (e) of Article 57 REACH**.**

Conclusion

Based on these elements, there is scientific evidence of probable serious effects to human health of resorcinol in relation to its thyroid-disrupting potential, which gives rise to an equivalent level of concern to those of other substances listed in points (a) to (e) of Article 57 REACH.

Registration dossiers submitted for the substance? Yes

Justification

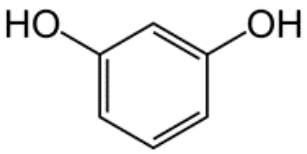
1. Identity of the substance and physical and chemical properties

1.1 Name and other identifiers of the substance

Table 1: Substance identity

EC number:	203-585-2
EC name:	resorcinol
CAS number:	108-46-3
IUPAC name:	benzene-1,3-diol
Index number in Annex VI of the CLP Regulation	604-010-00-1
Molecular formula:	$C_6H_6O_2$
Molecular weight range:	110.1
Synonyms:	1,3-dihydroxybenzene

Structural formula:



1.2 Composition of the substance

Name: resorcinol

Substance type: mono-constituent

1.3 Identity and composition of degradation products/metabolites relevant for the SVHC assessment

Not relevant for this dossier.

1.4 Identity and composition of structurally related substances (used in a grouping or read-across approach)

Not relevant for this dossier.

1.5 Physicochemical properties

Table 2: Overview of physicochemical properties

Property	Description of key information	Value	Reference/source of information
Physical state at 20°C and 101.3 kPa	-	solid	Dissemination site*
Melting/freezing point	Reliable secondary literature source	110°C	Dissemination site*
Boiling point	Reliable secondary literature source	277.5 °C at 1013 hPa	Dissemination site*
Vapour pressure	Extrapolated from experimental value	0.065 Pa at 25 °C	Dissemination site*
Density	Reliable secondary literature source	1.28 g/cm^3 at 20 °C	Dissemination site*
Water solubility	Experimental study	717 g/L at 25 °C and pH 7	Dissemination site*
Partition coefficient n-octanol/water (log value)	Measured data	0.8 at 20 °C	Dissemination site*

* https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/13740/1. Dissemination site was accessed on 17.12.2018

Conversion factors at 101.3 kPa and 20 °C are as follows: 1 ppm = 4.57 mg/m³; 1 mg/m³ = 0.219 ppm (WHO, 2006).

2. Harmonised classification and labelling

Resorcinol is covered by Index number 604-010-00-1 in part 3 of Annex VI to the CLP Regulation as follows:

Table 3: Classification according to Annex VI, Table 3.1 (list of harmonised classification and labelling of hazardous substances) of Regulation (EC) No 1272/2008

Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Spec. Conc. Limits, M-factors	Notes
				Hazard Class and Category Code(s)	Hazard statement code(s)	Pictogram, Signal Word Code(s)	Hazard statement code(s)	Suppl. Hazard statement code(s)		
604-010-00-1	resorcinol 1,3-benzenediol	203-585-2	108-46-3	Acute Tox. 4* Skin Irrit. 2 Eye Irrit. 2 Aquatic Acute 1	H302 H315 H319 H400	GHS09 GHS07 Wng	H302 H315 H319 H400	-	-	-

3. Environmental fate properties

Not relevant for the identification of the substance as SVHC in accordance with Article 57 (f) REACH due to its endocrine disrupting properties for human health.

4. Endocrine disruption relevant for human health

4.1 General approach

Resorcinol has been used as a medicinal ingredient in human dermatology since late in the 19th century to treat ulcerating skin lesions and has long been described to interfere with metabolism of the thyroid gland (Fawcet & Kirkwood, 1953). The analysis therefore concentrates on the effects related to the thyroid.

Definition of an endocrine disruptor

The WHO/IPCS (2002) definition of an endocrine disruptor (ED) is widely accepted:

"An endocrine disruptor is an exogenous substance or mixture that
- alters function(s) of the endocrine system
- and consequently causes
- adverse health effects in an intact organism, or its progeny, or (sub)populations."[5]

The European Commission's Endocrine Disrupters Expert Advisory group agreed in 2013 "*that the elements for identification of an endocrine disrupter were demonstration of an adverse effect for which there was convincing evidence of a biologically plausible causal link to an endocrine disrupting mode of action and for which disruption of the endocrine*

[5] Exact text quoted from WHO/IPCS (2002) definition but formatting of the text using bullet points added to emphasise major components of the definition.

system was not a secondary consequence of other non-endocrine-mediated systemic toxicity. Relevance of the data to humans should be assumed in the absence of appropriate data demonstrating non-relevance." (JRC 2013)

It is assumed in this report that a substance should fulfill the recommendations from the European Commission's Endocrine Disrupters Expert Advisory group outlined above in order to be identified as an endocrine disruptor, and available information has accordingly been assessed based on:

- Adverse health effects
- Endocrine mode of action (MoA)
- Biologically plausible causal link between adverse effects and endocrine MoA
- Human relevance

Selection and assessment of data

The assessment focuses on the investigation of effects related to the disruption of the thyroid function by resorcinol in humans and in toxicological models as well as on relevant information for the interpretation of data (e.g. toxicokinetics). Endocrine disruption relevant for the environment is currently further investigated by France in the context of Substance Evaluation under REACH (CoRAP 2019). Relevant data on environmental species are presented in section 5 to offer a comprehensive description of data on ED properties in vertebrates but are not evaluated in detail in this document.

The assessment is based on the information available in the registration dossier (update of 01/02/2019; public information from the dossier available on https://echa.europa.eu/registration-dossier/-/registered-dossier/13740) as well as in previous reviews: scientific evaluation of 12 substances in the context of endocrine disrupter priority list of actions (EC, 2002), assessment performed in the WHO/UNEP program (WHO, 2006), Conclusion document of Substance Evaluation by Finland (Tukes, 2017). In addition, a literature search targeted specifically on the identification of publications relevant for investigation of thyroid function was performed in Scopus using the search terms KEY (resorcinol) AND TITLE-ABS-KEY (toxicity OR thyroid). The latest update of the literature search was performed on 12 November 2019.

In accordance with ECHA guidance on "How to use alternatives to animal testing" (ECHA, 2016), studies were then considered for their relevance, reliability and adequacy.

Studies were first selected for their relevance and adequacy for the assessment of thyroid disruption. Prenatal and reproductive toxicity studies are included because thyroid disruption may have consequences on development.

With the exception of toxicokinetic studies, experimental studies in which resorcinol is used in a mixture (e.g. in hair dyes formulation) are excluded from the assessment as it is not possible to conclude whether the observed effects are due to resorcinol or to the other substances present in the mixture. Data included in this assessment are from testing of resorcinol. Data generated with substances expected to metabolise into resorcinol (e.g. resorcinol diacetate) are not included.

Most of the *in vivo* studies are performed using oral, dermal or inhalation exposures. In addition, some studies conducted by the subcutaneous route are also included. Subcutaneous administration bypasses the digestive and intestinal barrier and the enterohepatic first-pass metabolism as well as the skin barrier and metabolism. It is generally well accepted that unconjugated resorcinol is the biologically active form and subcutaneous administration may result in higher plasma levels of unconjugated resorcinol compared to a similar dose by oral route. However, other non-artificial routes of exposure

such as the dermal route or inhalation also bypass hepatic first pass. The subcutaneous route of exposure is considered relevant for MoA considerations and to support hazard identification

The other routes of administration used (intravenous, intraperitoneal) are anecdotal and the corresponding studies are considered only in relation to the analysis of the MoA.

The reliability of the selected experimental studies is assessed using ToxRtool. ToxRtool[6] was developed by the European Commission's Joint Research Center in 2009 (Segal *et al*., 2015) and builds on Klimisch categories by providing additional criteria and guidance for assessing the reliability of toxicological studies. It is applicable to various types of experimental data, endpoints and studies (study reports, peer-reviewed publications). ToxRtool scores 1 and 2 are defined as reliable without restrictions and reliable with restrictions, respectively. ToxRtool 3 is assigned as not reliable. In consequence they should not be used as key studies, but depending on the shortcomings of the study it may still be useful in WoE approaches or as supportive information. The ToxRtool score of each experimental study is justified in the corresponding summary tables by the description of possible limitations, when relevant.

All publications providing information on the effect of resorcinol on the thyroid in humans are included in the analysis. Most case reports relate to exposure to resorcinol on damaged skin. Alteration of the skin is expected to have an impact on the systemic availability of resorcinol as further discussed in section 6.3.2.1 in relation to the level of concern. However, a skin absorption potential exists (see section 4.3). In addition, one human case with intact skin displays the same effects supporting that these data are relevant for the assessment of the intrinsic properties of resorcinol in general and its endocrine disrupting properties in particular. ToxRTool (or Klimisch) assessment is not relevant for human data. ECHA's guidance on information requirements mentions in Chapter R.4: Evaluation of available information (2011) that the strength of the epidemiological evidence for specific health effects depends, among other things, on the type of analyses and on the magnitude and specificity of the response. Other characteristics that support a causal association are presence of a dose-response association, a consistent relationship in time and (biological) plausibility. Many human data available for resorcinol are case reports and some of these parameters are not relevant (i.e. dose-response). Each study was however described with all available and relevant information and assessed for its potential limitations and contributions in the analysis.

All studies are qualitatively weighted based on expert judgement to produce a conclusion on the selected adverse effects and their thyroid-disrupting MoA. Human data are analysed on one hand. On the other hand, experimental data are compared to each other with specific consideration given to the routes of exposure in particular.

The conclusion of the weight of evidence (WoE) analysis is based on the combination of human and experimental *in vivo* and *in vitro* data. The database includes some studies performed decades ago and the reliability of these studies may be lower and inadequate to be considered as key studies due to poor reporting in particular. These studies have been included in the WoE analysis, in line with ECHA's Practical Guide: How to use alternatives to animal testing (ECHA 2016) that concludes that "these studies could be adequate within a weight of evidence approach or as supporting studies". ECHA's practical guide (2016) further defines that "The weight of evidence approach commonly refers to combining evidence from multiple sources to assess a property under consideration". As discussed in the guide, the WoE approach is beneficial when the information from each source individually may be regarded as not sufficient and when several available studies give conflicting results. It also emphasises that "Expert judgement is vital in the construction and appraisal of the WoE package, namely when considering the reliability,

[6] https://eurl-ecvam.jrc.ec.europa.eu/about-ecvam/archive-publications/toxrtool

relevance and adequacy, integrating and comparing different pieces of information and assigning a weight to each piece of data."

4.2 Background information on thyroid hormones and TPO

Thyroid hormones, triiodothyronine (T3) and thyroxine (T4), control major functions such as metabolism, growth, and brain development and function. Thyroid hormones play an important role in foetal and postnatal development and in particular in the development of the central nervous system.

In humans, the major form of thyroid hormone in the blood is thyroxine (T4), which has a longer half-life than T3 (T4:T3 ratio of approx. 14:1, Mortoglou 2004). T3 is the main form activating thyroid hormone receptors and T4 is converted to the active T3 in the liver and within some tissues including brain by 5'-deiodinases (Peeters & Visser, 2017). More than 99% of T3 and T4 are bound to plasmatic transport proteins: thyroxin binding protein (TBG), transthyretin (TTR) or albumin (Alshehri *et al*., 2015; Janssen & Janssen, 2017). Individual levels of thyroid hormones are regulated in a narrow range (Gilbert *et al.,* 2012). They are produced in the follicular cells of the thyroid in response to thyroid-stimulating hormone (TSH). Synthetised in the pituitary gland, TSH is regulated by T3 and T4 via a negative feedback loop.

T3 and T4 are formed when tyrosine residues of thyroglobulin (Tg) are iodinated by the enzyme thyroperoxidase (TPO) and the iodinated protein is cleaved. Tg is produced in the endoplasmic reticulum of the thyroid follicular cells and is stored in the follicles mostly as colloid. Iodide (I^-) is actively pumped into the cell by a sodium-iodide (Na/I) symporter (NIS). In the colloid, TPO catalyses iodide oxidation into iodine (I^0) in the presence of hydrogen peroxide, regulates nonspecific iodination of tyrosyl residues of thyroglobulin to form TH precursors monoiodotyrosine (MIT) and diiodotyrosine (DIT) and modulates coupling of these iodotyrosyl residues. Thyroxine (T4, derived from DIT-DIT coupling) and triiodothyronine (T3, derived from MIT-DIT coupling) are subsequently liberated from thyroglobulin by proteolysis in the thyroid follicular cells and released into systemic circulation. Efflux of thyroxine and triiodothyronine from follicular cells into the blood appears to be largely through monocarboxylate transporters (MCT) 8 and 10 (Paul Friedman *et al*., 2016, US EPA, 2019; Groeneweg *et al.,* 2020).

NIS and TPO are therefore the two key enzymes in the *de novo* synthesis of thyroid hormones and TSH stimulates T3 and T4 production and secretion through stimulation of the different steps of TH synthesis including the gene expression of NIS and TPO.

Various transcripts of the TPO gene exist in humans as a consequence of differences in splicing (Gardas *et al.,* 1997; Ferrand *et al*., 2003). The level of expression of some isoforms is associated with some pathologies (Gardas et al., 1997; Le Fourn, 2004). It may explain, partly, the heterogeneity of the TPO purified from human thyroid glands. The role and activity of the different isoforms in the process of thyroid hormone synthesis and the true chemical structure of the thyroperoxidase present in normal human thyroid tissues needs to be further investigated.

In addition, more than 70 distinct mutations of the TPO gene have been published worldwide in humans. An epidemiology study showed that the frequency of TPO deficiency was 1 in 66,000 among the general Dutch population. The classical hallmark of TPO deficiency is total iodine organification defect. A minor subset of TPO mutation carriers have partial iodine organification defect, though the frequency of the phenotype remains unknown (Narumi *et al.*, 2017).

In humans, hypothyroidism is a condition characterized by insufficient THs. Deficiency of THs can be moderate or severe. It is called overt or clinical hypothyroidism when TSH is above the upper limit of normal and free T4 (FT4) is lower than the range of reference. Subclinical hypothyroidism is defined by TSH level above the upper limit of normal but FT4 is within the reference range. Isolated hypothyroxinemia is defined as a normal maternal TSH concentration and FT4 concentration is lower than the range of reference (López-Muñoz *et al.,* 2019).

No such reference ranges are established in model animal species. Furthermore, FT4 is rarely measured in animal experiments.

4.3 Toxicokinetics (absorption, metabolism, distribution and elimination)

Data investigating the toxicokinetic of resorcinol are available by oral, subcutaneous and dermal routes and are summarised respectively in tables 4, 5 and 6 below.

Oral route

Resorcinol is well absorbed by gavage. When administered in water by gavage in Garton & Williams (1949), 77% of the administered dose is absorbed in rabbits in 24h. Similar observations are reported by Deichmann & Thomas (1943). When administered in corn oil by gavage, absorption reaches 92 to 95% in rats in 24h (Kim & Matthews 1987).

Resorcinol is rapidly metabolised and excreted in the urine (90-93% in 24h) primarily as monoglucuronide conjugate (Garton & Williams, 1949; Kim and Matthews, 1987). Minor metabolites include a monosulphate conjugate, a mixed sulphate-glucuronide conjugate, and a diglucuronide conjugate. Parent resorcinol is also detected in urine (1.2 to 4.6%, Kim and Matthews 1987).

In vitro, half-lives of 55 and 22 min respectively are measured after incubation of resorcinol with human hepatic S9 or primary human hepatocytes (Eilstein *et al*., 2020). Glucuronide is formed. It supports an efficient first-pass metabolisation in the liver.

In rats, free resorcinol is detected in plasma 30 min to 2 h after gavage but not later, from a dose of 80 mg/kg/d resorcinol in a 13-week study (unpublished study report, 2004a). In a drinking water study, free resorcinol is detected in the blood of some animals exposed at the highest dose of approximately 245/295 mg/kg/d (unpublished study report, 2005a).

Variations across time were not investigated in this study and it is not possible to compare the kinetic and total systemic exposure to free resorcinol between gavage and oral administration in drinking water.

A rapid total clearance rate of 138.8 ml/min is estimated using a physiologically-based pharmacokinetic model integrated with a pharmacodynamics model (PBPK/PD) simulating oral ingestion in humans (Leonard *et al.,* 2016).

Subcutaneous routes

Important metabolisation (84% of glucuronide) and urinary excretion (98% of applied dose) are observed after 24h in rats after a single subcutaneous dosing of 10 mg/kg (Merker *et al.* 1982). A 90% plasma clearance is observed within 2h with biphasic half-life of 18-21 min and 8.6-10.5 h.

Dermal route

It is difficult to predict dermal absorption of resorcinol based on its physicochemical properties. According to ECHA's guidance R.7c (section R.7.12.2.1 Derivation of TK information taking into account a Basic Data Set, June 2017):

- Water solubility of resorcinol (717 g/L) is above the range (0.1 to 10 g/L) reported to have a moderate to high potential for dermal absorption.
- While log Kow value below 0 are expected to limit penetration, dermal absorption is favoured between 1 and 4, particularly if water solubility is high. With a log Kow of 0.8 and a high water solubility, it cannot be concluded whether dermal absorption would be expected to be low or high.
- With regard to molecular weight, guidance indicates that less than 100 favours dermal uptake while above 500 the molecule may be too large. Resorcinol is a small molecule (MW 110) and its size is not expected to prevent its absorption.

Therefore, no clear conclusion can be made on dermal absorption based on physicochemical properties.

Based on data from the REACH registration dossier[7], it is noted that acute toxicity is higher by oral route (LD_{50} of 510 mg/kg in rats) than by dermal route (LD_{50} of 2830 mg/kg in rabbits[8]). These data indicate that the dermal absorption is expected to be lower than absorption by oral route (factor of 5.5 from these data; considering an oral absorption of 85%, it would indicate a dermal absorption of approx. 15%). Data were however obtained in different species and LD_{50} determination is a rough statistical determination of the toxicity potential, which limits the interpretation of this comparison.

In a human volunteer study (Yeung *et al*., 1983), no resorcinol nor conjugates were detected in plasma after up to 4 weeks of dermal application of resorcinol in a hydroalcoholic vehicle on intact skin. The blood sampling time was not specified. However, resorcinol is detected in urine as conjugates after 2 weeks of dermal application. Up to 2.87% of the applied dose is absorbed, corresponding to a penetration flux of 0.37 µg/cm²/h. A variation of a factor of 6 is observed between the lower and higher individual excretion from the 3 subjects. A similar flux is observed *in vitro* (0.86 µg/cm²/h, 5% of dose absorbed).

In hair dye formulations, radioactivity from resorcinol is detected in the urine of human volunteers and Rhesus monkeys after a hair-dyeing procedure with 1.225% resorcinol in a study with some limitations (incomplete collection of urine in Rhesus monkeys). The estimated urinary excretion is 0.076% of the applied dose in humans (Wolfram & Maibach, 1985). Low absorption is similarly reported *in vitro* on human skin exposed to hair dye formulations containing 0.61% resorcinol (0.05 µg/cm²/h, Dressler, 1999) or 1.26% resorcinol (1.04-2.95 µg/cm²/h and 0.32-0.82%, OECD guideline 428, Unpublished study report, 2005b).

A rate of 12 µg/cm²/h can be calculated from another *in vitro* study on human skin with 10% resorcinol in water (Roberts *et al*., 1977) in which the integrity of the skin sample was not confirmed.

A series of recent *in vitro* studies using phosphate buffered saline (PBS) as a vehicle report much higher absorption rates. Genies *et al.* (2019a) report that 46.7% and 50.9% of the

[7] https://echa.europa.eu/registration-dossier/-/registered-dossier/13740/7/3/1, accessed on 5 May 2020

[8] Half of the animals had abraded skin and half non-abraded skin. However, no difference in sensitivity was observed between animals with abraded and non-abraded skin with 0/4 deaths at the dose of 2000 mg/kg and below and 4/4 deaths at the next higher dose of 3980 mg/kg and above.

applied dose (0.96 and 0.26 µg/cm²) is detected in the receiving media after 24h of incubation on fresh human abdominal skin (4 donors) and on pig skin, respectively. In human skin, 90% of the radioactivity present is attributed to metabolites with glucuronide conjugate being the major metabolite (33.7% of total dose) and sulfate conjugate the minor metabolite (8.2%). Parent resorcinol is however also present and amounts to 4.8% of the applied dose. A similar absorption rate is reproduced in a follow-up study (Genies *et al*., 2019b) that investigated the kinetics of absorption and metabolism over 5 concentrations from 0.9 to 96.0 µg/cm². The proportion of applied dose detected in the medium increased progressively over time: depending on the test concentrations, 0.37 to 6.42% was measured after 2 h and consists of parent resorcinol only. It reached 45.8 to 49.01% after 24 h of incubation and consisted of 23 to 90% of metabolites (10 to 77% of parent resorcinol), indicating a progressive metabolisation. The percentage of metabolites formed was lower at higher concentrations and indicate a saturation of metabolisation at higher concentrations. The dose does not affect the speed of absorption but saturation of metabolisation is observed. Using Episkin™, only 1% of the dose was metabolised to glucuronide after 2 h. Eilstein *et al*. (2020) also did not detect metabolisation in Episkin™ after 2 h. Using abdominal skin tissue in a study compliant with OECD 428 protocol, Hewitt *et al.* (2020) determined a dermal delivery of 74.2±8.8% of the applied dose after 24h. The use of frozen tissue is not expected to impact the overall absorption rate as skin integrity was checked experimentally, as required by the OECD guideline. However, the metabolic capacities of the skin may have been altered. For this reason, the study is not informative on the proportion of absorbed resorcinol available as resorcinol or as conjugated metabolites. It is noted that the recent studies tested low concentrations and the difference with previous studies may reflect that small concentrations are proportionally better absorbed than higher concentrations.

There is no toxicokinetic study that investigated the absorption and metabolism of resorcinol by inhalation.

Overall, resorcinol is well absorbed by the oral route (77-95% after 24h by gavage). A low dermal absorption rate has been reported in a human volunteer study (up to 3% after 24 h) and in several *in vitro* studies on human skin (0.1-5% after 24h) using hydroalcoholic vehicle or hair-dye formulations. However, a series of recent *in vitro* data consistently report a much higher absorption of low concentrations of resorcinol in PBS (50 to 70% after 24h). Comparison of acute dermal and oral toxicities indicates 5 times lower internal levels by dermal route. Common metabolites are formed by oral, subcutaneous and dermal routes with glucuronide as the major metabolite and sulfate as a minor metabolite. In the skin, no metabolisation was observed after 2h and depending on test concentrations, 10-77% of resorcinol is still present as parent resorcinol after 24h *in vitro*. In hepatic cells, metabolisation is efficient (half-life of 22 to 55 min). Fast systemic metabolisation and excretion is observed but small amounts of free resorcinol (1.2-4.6%) are detected in urine after gavage administration.

The available data do not show accumulation in any organ or tissue, including the thyroid gland.

Table 4: Overview of toxicokinetic studies by oral route

ORAL ROUTE Study	ToxRtool score	Results	Reference
In vivo, oral route (specific mode of administration not known) Rabbits Single dose, 400 mg/kg	Secondary source. ToxRtool 4.	Moderate increase in glucuronic acid and organic sulfate conjugates observed in the urine collected during the following 3 days.	Deichmann & Thomas (1943) (quoted from Brandt 1986)
In vivo, oral route (gavage) Rabbits (giant chinchilla) Exp I: n=3 fed by stomach tube with 100 mg/kg resorcinol dissolved in water Exp II: n=6 fed with 1 g/animal. 24-h urine collected Exp II: n=6 fed with 6 g/animal. 24-h or 48-h urine collected	ToxRtool 2 Limitations: protocol for urine collection not described.	Exp I: 13.5% excreted as monosulphate, 52.0% as monoglucuronide Exp II: 11.4% of free resorcinol excreted in 24-h urine; combined + free = 77% Exp III: no trace of oxidised metabolites in urine	Garton & Williams, 1949
In vivo, oral route (presumably gavage) F344 rats (n=3/sex/dose) Single oral dose of 112 mg/kg or 225 mg/kg [C^{14}]-resorcinol in corn oil, or 225 mg/kg/d for 5 consecutive days	ToxRtool 1	At 112 mg/kg, 90.8% (males) to 92.8% (females) excreted in urine and 1.5-2.1% in faeces within 24 hours and less than 0.1% as CO_2. After bile duct cannulation and iv administration of 11.2 mg/kg, amount excreted in bile duct was higher than faeces excretion after oral administration and shows enterohepatic circulation. Remaining C^{14} activity in blood, liver, skin, fat, muscle, large intestine. No indication of bioaccumulation in thyroid. Major metabolite (about 65%) is a glucuronide conjugate. Minor metabolites include a monosulphate conjugate, a mixed sulphate–glucuronide conjugate, and a diglucuronide conjugate. Greater proportion excreted as sulphate conjugate in females and as diconjugate (both sulphate and glucuronide groups) in males. Free resorcinol amount in urine after 24 hr was respectively 1.2% and 1.3% in males and females at 112 mg/kg and 4.6 and 2.2% at	Kim & Matthews 1987

ORAL ROUTE Study	ToxRtool score	Results	Reference
		225 mg/kg. Comparable results after repeated administration.	
In vivo, oral route (gavage) OECD 408 – 13-week study Sprague-Dawley rats (n=3/sex/group) Daily gavage to doses of 40, 80 or 250 mg/kg/d for 13 weeks Blood samples taken at day 1 and week 13 at 0.5, 1, 2, 4, 8, and 24 hours post dosing. LOQ: 0.5 µg/ml	ToxRtool 1	Day 1: plasma levels of (free) resorcinol quantifiable at least for two animals at all time points and all 3 doses. Plasma levels generally increased rapidly with a first maximum Cmax at 0.5 – 2 hours. In some cases, a second Cmax was seen at 8 and/or 24 hours. AUC and Cmax were not dose-dependent. Mean concentration remained stable over the 24 hour-period at 40 and 80 mg/kg/day, which may suggest enterohepatic recycling. Week 13: plasma levels quantifiable only at 0.5 to 2 hours for the 80 and 250 mg/kg/d groups. Cmax were generally higher and AUC generally lower than at day 1. Cmax and AUC increased with doses in a supra linear manner. At 250 mg/kg, the Cmax was 2.37 and 9.29 µg/ml at 0.5 h and the AUC 10.9 and 27.5 µg.h/ml in males and females, respectively. Inter-animal variability was considered low to moderate by the study authors.	Unpublished report 2004a
In vivo, oral route (drinking water) OECD 416 – 2-generation study Sprague-Dawley rats (n=7 to 10 /sex/group) Exposure levels were 0, 120, 360, 1000 and 3000 mg/L resorcinol in drinking water. Mean compound consumption in F1 animals were respectively 11, 33, 93 and 245 mg/kg/d in males and 16, 41, 126 and 295 mg/kg/d in females. Blood samples taken in F1 after 143 to 155 days of dosing and as soon as logistically possible after	ToxRtool 1	Blood (free) resorcinol levels (116 to 621 ng/mL) detected in some (3/20, 1 M and 2 F) animals in the 3000 mg/L group (exposed to approx. 245 mg/kg/d in males and 295 mg/kg/d in females). Levels below LOQ in other animals.	Unpublished report 2005a (Welsch, 2008a)

ORAL ROUTE Study	ToxRtool score	Results	Reference
one of the two peaks during the dark-phase water consumption by rats (between 6:15 and 7:15 am; end of dark phase: 6:00 am). LOQ: 100 ng/ml			
PBPK/PD model simulating oral ingestion	Not relevant	Total clearance rate estimated in humans based on PB/PK modeling: 138.8 ml/min	Leonard *et al.,* 2016
In vitro Human hepatic S9 fractions (pool of 200 donors) incubated with 5 µM resorcinol for 2 h Primary human hepatocytes (pool of 5 donors) incubated with 1 µM resorcinol for 180 min.	ToxRtool 1	Half-life with human hepatic S9: 55 min Half-life with primary human hepatocytes: 22 min Glucuronide metabolite formed.	Eilstein 2020

Table 5 : Overview of toxicokinetic studies by subcutaneous route

SUBCUTANEOUS ROUTE Study	ToxRtool score	Results	Reference
In vivo, subcutaneous route Sprague-Dawley rats (males) Single subcutaneous dose of 10, 50 or 100 mg/kg [C^{14}]-resorcinol in aqueous solution	Secondary source ToxRtool 4	98% of the applied dose excreted via urine and 1% via faeces, mainly as glucuronide conjugate (84%) within 24 h after dosing with 10 mg/kg. $_{14}C$ activity rapidly distributed in muscles, kidneys, liver, without indication of bioaccumulation. Approx. 90% of clearance in plasma within 2 h post-administration. Elimination biphasic with half-lives of 18–21 min and 8.6–10.5 h. Distribution profile 24 hours after 14 or 30 days exposure to 100 mg/kg/d was similar to that after single administration.	Merker *et al*. 1982 (quoted from WHO 2006 and EFSA 2010)

Table 6: Overview of toxicokinetic studies by dermal route

DERMAL ROUTE **Study**	**ToxRtool score**	**Results**	**Reference**
In vivo studies			
Human volunteer study (*in vivo*) Four healthy males with intact skin (aged 18 +). Three in the treatment group and one control (vehicle). 20 ml of 2 % resorcinol in hydroalcoholic vehicle applied twice daily, 6 days/week for 4 weeks (to the face, shoulders, upper chest and upper back) Daily dose of 12 mg/kg/d (0.30 mg/cm²) Blood samples drawn at day 0, and at weeks 1, 2, 3, and 4 after the initiation of treatment (time not stated). 24-hour urine specimens collected 2 and 4 weeks after initiation of treatment. Samples assayed by GC/MS for free resorcinol and/or its conjugates (glucuronide or sulphate); blood chemistries (SMAC 24) and thyroid functions (T3, T4, T7 and TSH) were also measured. LOD for resorcinol: 0.1 µg /ml.	ToxRtool 2 Limitations: ▪ Single blood sampling and time after exposure not known. Depending on kinetic, exposure may have been missed. ▪ 24-hr urine collection incomplete at 4 weeks These limitations are considered to affect some but not all the results of the study.	No detectable levels of free resorcinol or its conjugates in plasma. No detectable free resorcinol in urine sample. Resorcinol conjugates measured at 2 and 4 weeks: - 2 weeks: 24-h excretion of 3 752 to 22 982 µg (**0.47 to 2.87% of applied dose – average 1.64%)** - 4 weeks: quantification not appropriate due to incomplete collection of urine. Skin penetration flux: **0.37 µg/cm²/h** (calculated from subject with maximal excretion rate). No effect on thyroid hormones or blood chemistry.	Yeung *et al*. 1983
In vivo experimental study Female hairless Wistar rats (n=12) Radioactive resorcinol added to a hair-dye formulation (with 1.5% resorcinol) applied for 30 min.	ToxRtool 2 Limitations: ▪ Results from the main test (measure of skin penetration) not reported for resorcinol.	No radioactivity detected in the thyroid of animals sacrificed 4 days after exposure. Only traces were detected in the liver (2.2 10^{-3} µg)	Tsomi & Kalopissis 1982
Human volunteer and experimental study (*in vivo*) Three human volunteers with urine collected for 24 h (and later if required) after one hair dyeing procedure Three Rhesus monkeys with urine samples collected 6, 12, 24h and at 24-h interval for 7 days after one hair-dyeing procedure. Radioactive resorcinol added to a hair-dye formulation with 1.225% resorcinol, applied for 23 to 28 min.	ToxRtool 3 Limitations: ▪ applied dose of radioactive resorcinol not given, ▪ incomplete collection of urine in Rhesus monkeys ▪ poor reporting of results (only total dose excretion provided) Used in a WoE	Human: Half-life of urinary excretion: 31 h Total dose excretion: **0.076±0.03%** Rhesus monkey: Half-life of urinary excretion: 31 h Total dose excretion: **0.177±0.03%**	Wolfram & Maibach 1985

DERMAL ROUTE Study	ToxRtool score	Results	Reference
	approach as supporting study		
In vitro studies			
Human abdominal skin from one subject, separated by exposure to ammonia fumes for 30 min Resorcinol concentration: 10% in water. Each study performed at least in duplicate	ToxRtool 3 Limitations: ▪ only 1 subject, ▪ samples used for several experiments, ▪ no information on duration and volume of application ▪ method of separation of the skin may have impacted its integrity Used in a WoE approach as supporting study	Lag time: 80 min Penetration of a cumulative amount of approx. 0.05 mg/cm² after 250 min (**approx. 12 µg/cm²/h**). Permeability coefficient: 0.00024 cm/min	Roberts *et al*. 1977
Human full-thickness skin (excised) 0.39 mg/cm² of radiolabelled resorcinol in hydroalcoholic vehicle	ToxRtool 1	Flux: **0.86 µg/cm²/h** Corresponding to 5% of the applied dose after 24h	Yeung *et al*. 1983
Human skin from 6 donors (16 replicates) 0.61% resorcinol in a representative hair dye formulation	Secondary source ToxRtool 4	Plateau in receptor fluid concentrations between 24 and 48h, as reflected by cumulative absorption values of 1.17 and 1.30 µg/cm², respectively (corresponding to **0.05 and 0.03 µg/cm²/h**, respectively.	Dressler 1999 (quoted from WHO 2006)

DERMAL ROUTE			
Study	**ToxRtool score**	**Results**	**Reference**
Human split-thickness skin membranes Exposure regime: 0.5 hours then wash, samples taken at 24 hrs post-exposure. Doses/conc.: Actual Resorcinol concentration in formulation (% w/w): 2.55 (Oxidative); 2.52 (Non-Oxidative). Actual Resorcinol concentration in Test Preparation (% w/w): 1.26 (Oxidative); 1.27 (Non-Oxidative). Actual application rate of Test Preparation (mg/m^3): 21.08 (Oxidative); 20.07 (Non-Oxidative). OECD Guideline 428	Secondary source (quoted as Reliability 1 in Tukes 2017)	<u>Oxidative preparation</u> The total recovery, dislodgeable dose, unabsorbed dose, absorbed dose and dermal delivery were 252.02, 248.92, 250.97, 0.84 and **1.04 µg equiv./cm²**, respectively. <u>Non-Oxidative preparation</u> The total recovery, dislodgeable dose, unabsorbed dose, absorbed dose and dermal delivery were 249.57, 242.65, 246.62, 2.10 and **2.95 µg equiv./cm²,** respectively. **Percutaneous absorption rate: 0.32 % (oxidative) and 0.82 % at 24 hours (non-oxidative)**	Unpublished report 2005b (quoted from Tukes, 2017)
Human abdominal skin (4 donors) and pig ear skin 0.96 and 0.26 µg/cm², respectively of radiolabelled resorcinol in phosphate buffered saline Skin integrity confirmed by histological analysis of non-treated explants.	ToxRtool 1	Applied dose detected in culture media after 24h: - **46.7±0.7**% with human skin (4.8% as free resorcinol, 33.7% as glucuronide, 8.2% as sulfate conjugate) - **50.9±1.2%** with pig skin (1.8% as free resorcinol, 48.6% as glucuronide, 0.5% as sulfate conjugate)	Genies *et al*. 2019a
Human abdominal skin (4 donors) exposed to 0.9 to 96.0 µg/cm² resorcinol in phosphate buffered saline for up to 24 h Skin integrity was confirmed by histological analysis of non-treated explants. EpiSkin™ S9 from reconstructed human epidermis models incubated with 550 µg resorcinol for 2 h	ToxRtool 1	<u>Human explants:</u> Applied dose detected in the medium after 1 h: 0.14 to 2.92% Applied dose detected in the medium after 2 h : 0.37 to 6.42%, depending on test concentrations No metabolite detected Applied dose detected in the medium after 18 h: 29.5 to 37.7% Applied dose detected in the medium after 24 h: **45.8 to 49.0%**	Genies *et al*. 2019b

DERMAL ROUTE Study	ToxRtool score	Results	Reference
		Metabolites represent 23 to 90% EpiskinTM: 1% of the dose metabolised (glucuronide) after 2 h.	
Human abdominal skin (4 donors; 3 replicates/donor; 1 cm²) exposed to 100 µg/cm² resorcinol in phosphate buffered saline for up to 24 h using non-occluding conditions Integrity of skin confirmed by measuring trans-epidermal water loss. OECD Guideline 428	ToxRtool 1	Dermal delivery: **74.2±8.8%** of applied dose in epidermis (3%), dermis (1.1%) and receptor fluid (70.1%) 5.23±3% present in stratum corneum strips Cumulative amount in receptor fluid was 8.92±11.76 µg after 2 hr, 43.90±17.53 µg after 8h and 68.60±11.19 µg after 24h. As assay was conducted using frozen skin, this is expected to reflect the absorption of unmetabolised resorcinol but it does not inform on the proportion of absorbed resorcinol available as resorcinol or as conjugated metabolite.	Hewitt *et al*. 2020
EpiSkin™ S9 incubated with 5 µM resorcinol for 2 h	ToxRtool 1	Resorcinol was concluded as stable over 2 h.	Eilstein *et al*. 2020

4.4 Adverse effect of resorcinol on thyroid function

4.4.1. Human data

Case reports

A number of case reports related to repeated exposure to resorcinol are published in the literature and are presented below by chronological order of publication.

Bull & Fraser published a report of 3 cases in 1950.

Case 1 was a woman (60-year old, 112.5 kg) with cardiac insufficiency and bilateral ulceration of the legs who applied a resorcinol ointment to the legs (dose and duration not

known). She developed in 7 months an enlargement of the neck with dysphagia and a hoarse voice, weight gain (+22 kg), coarse hair, dry skin, myoedematous appearance. During this period, the only medication received is digitalis folia gr. 1 twice a day and applications of resorcinol ointment to the legs. Protein-Bound Iodine (PBI[9]) was 1 µg/100 ml (normal: 4-8 µg/100 ml**). Urinary excretion of radioactive iodine was extremely low (7.6% in 48h) suggesting an avid uptake by thyroid**. The patient died from heart failure. Necropsy reveals a **thyroid gland about four times the normal size** (130 g). Thyroid was pale and fleshy with gross parenchymatous hyperplasia, **with small and large follicles, mostly depleted of colloid**. The authors suggest that the **absence of lymphoid follicles indicates a response to a goitrogen**.

Case 2 was a woman (50-year old, 75 kg) in good general health except varicose veins and ulcer on both legs. She applied an ointment with 12% resorcinol in glycerin, paraffin, and lanolin to the legs daily for 2 years (114-227 g/wk, equivalent to 1954-3891 mg/d or 26-52 mg/kg/d). The patient developed tiredness with backache for 2 months, tinnitus and deafness for 6 weeks, dyspnea for four weeks, sensitivity to cold, puffiness, dry skin, hoarseness and slight cough for 2 weeks. She had a typical myxoedematous appearance and palpation of the neck revealed a **very soft, diffusely enlarged thyroid**. PBI was under 1 µg/100 ml. **Urinary excretion of radioactive iodine was very low (6% in 48 h) and thyroid uptake was high (62% at 73h). Thyroid biopsy showed a hyperplastic gland with small and medium-sized follicles empty of colloid and lined by high cuboidal and columnar epithelium**. Restoration of normal general health was obtained after cessation of application of resorcinol ointment without any medication. The authors indicate that measurements of thyroid function during a further fortnight with application of the ointment and a further fortnight after **withdrawal confirmed the anti-thyroid effects of the ointment**.
Case 3 was a woman (50-year old, 70 kg) in good general health except for a varicose ulcer on the left leg for 7 years. She applied 450 g/wk of an ointment with 4% resorcinol to the leg for 3 years (equivalent to 2570 mg/d or 37 mg/kg/d). The patient noticed swelling of the neck for 2 years, experienced an attack of jaundice 3 years and 7 months previously and was pale, **depressed** and lethargic for 3 years. **The thyroid was diffusely enlarged** (approx. 100 g). Urinary excretion of radioactive iodine was very low (9.9% in 48 h). Five weeks after cessation of use of the ointment, considerable improvement in symptoms was noted (lethargy and depression lessened, skin warmer and softer, anemia improved). **Thyroid biopsy showed a hyperplastic gland with small and large follicles. The majority contained colloid, mostly showing peripheral vacuolation.** The authors mention suggesting a phase of recovery in a **goitrogen-induced hyperplasia**. Seven week after cessation of resorcinol treatment, PBI was normal (4.9 µg/100 ml). Restoration of normal general health was obtained after cessation of application of the resorcinol ointment without any medication. The authors indicate that measurements of thyroid function during a further fortnight with application of the ointment and a further fortnight after withdrawal confirmed the **anti-thyroid effects of the ointment**.

One case was reported by Hart & Maclagan in 1951. A woman (45-year old) with ulcers of the legs for 17 years developed after puerperal septis and femoral thrombosis, used daily dermal application of ointment with 4% resorcinol for approximately 12 years. Other ingredients of the ointment were lanolin (39.3%), soft paraffin (46.7%), zinc oxide (10.0%) and cresol (0.002%). She experienced **enlarged thyroid** and hoarse voice for 5 years. For 2 years, acceleration of thyroid enlargement is reported as well as an appearance suggesting anemia, **impairment of memory and powers of concentration** and thin hair. On examination, thyroid was markedly and evenly enlarged, face was pale

[9] PBI test indirectly assesses thyroid function by measuring the concentration of iodine bound to proteins circulating in the bloodstream. Thyroid hormones are normally transported in the bloodstream by carrier proteins. In the PBI test, these carrier proteins are precipitated from the blood, and the quantity of bound iodine is measured. The determination of PBI has been found to parallel quite closely the concentration of the active thyroid hormone in the blood stream. (Encyclopaedia Britannica, accessed on 01/03/2019)

and puffy, hair was thin and scanty, dry skin, husky voice, poor memory and concentration were noted. Uptake of radioactive iodine was well above normal. Minimal thyroid enlargement was present 8 months after withdrawal of resorcinol from the ointment and puffiness disappeared. The patient died from worsening of ulcerations into extensive burns.

One case was reported by Hobson (1951). A man (54-year old) with varicose veins with ulceration for 8 years used daily dermal application of ointments containing resorcinol (4% then 12 %) to varicose leg ulcers for 9 months. **The patient showed clinical symptoms of goitre (enlarged thyroid, dry puffy skin)** and myxoedematous face and complained of weakness, palor, lassitude, dyspepsia for 4 months and swelling of the neck and hoarse voice for 2 weeks. **PBI was low** (<0.5 µg/100 ml). Withdrawal of the ointment for 6 weeks resulted in improvement of well-being and reversal of myxoedema. The goitre was smaller but palpable. PBI was normal (5.5 µg/100 mL). Reapplication of the resorcinol ointment resulted in diminished thyroid uptake of radioactive iodine and increased urinary excretion.

One case was reported by Thomas & Gisburn (1961). The patient was a woman (59-year old) with ulceration of the leg resulting from phlebitis 33 years before and that used daily dermal application of ointment with 12.5% resorcinol and 12.5% glycerin in a soft paraffin base for 13 years and up to 700 g every 10 days in the 2 last years (equivalent to 875 mg/d). She suffered from myxoedema (extreme loss of energy, generalised oedema) for the last 6 years and **her symptoms were relieved by treatment with thyroid tablets.** The patient complained of loss of energy, was pale with moderate goitre and although still obese showed signs of recent loss of weight. In addition, her ears had a bluish-grey discoloration and triangular brown patches with their bases towards the cornea could be seen on the sclerotics. A black deposit was seen on the ulcers and surrounding skin and dark urine was observed. There were signs of osteoarthritis in the knees. These symptoms are consistent with the diagnosis of ochronosis, a syndrome caused by the accumulation of the phenolic acid homogentisic acid in connective tissues. Her urine contained 2.1% of a mixture of resorcinol glucuronide and resorcinol monosulphate. Free resorcinol was not detected. She died from extensive mesenteric thrombosis with gangrene of the small intestine. **The thyroid was enlarged**.

Guinet *et al.* (1967) report another case. A woman (39-year-old) had been suffering from bilateral phlebitis since the age of 20 and varicose veins of both legs were complicated by chronic oedema and ulcerations. She applied an ointment containing 2 % resorcinol to damaged skin for leg ulcers for about one year. **After a few months of treatment, a hyperplastic parenchymatous goitre (hyperplasia of thyroid) with discrete hypothyroid symptoms** (pale face, dry skin, cryophobia, modification of the voice) was described. Upon cessation of the application of resorcinol and treatment with liothyroxine (100 µg/d) for one month, the goitre reduced in volume. Two months after cessation, clinical signs of hypothyroidism had disappeared but uptake of radioactive iodine was still elevated. **Reduction to a non-vascular goitre and flattening of the radioactive iodine uptake curve was observed after 8 months, together with administration of a sufficient dose of liothyroxine (up to 200 µg/d)**. The authors concluded that **resorcinol is the antithyroid factor, by blocking at least partially organification of iodine**. An individual susceptibility of thyroid is also hypothesised.

Two cases are reported in Berthezene *et al.* (1973).

A woman (68-year old, overweight with pre-diabetic condition, 96 kg) had a 5-year history of treatment with 7 g/day resorcinol (73 mg/kg/d). **Goitre and clinical signs of hypothyroidism** (pale yellow and puffy face, hoarse voice, cold extremities) **had developed 1 year after the start of treatment**. Moderate hypothyroidism was confirmed by biological data: uptake of radioactive iodine by thyroid was 37% for the 1st hour and 48% for the 2nd hour and was quickly blocked by *per os* administration of

perchlorate (indicating that NIS was functional). Plasmatic iodide was elevated (3 µg/100 mL) and the uptake by thyroid of stable iodine was elevated (157.1 µg/h). One month after cessation of resorcinol treatment, the volume of the goitre was reduced and clinical signs of hypothyroidism had stopped. Uptake of radioactive iodine by thyroid was still high (50 and 63% for the 1st and 2nd hours) but was not modified by perchlorate administration. The uptake by thyroid of stable iodine was decreased to 8.7 µg/h (normal value 2.4±1.3 µg/h). The authors hypothesised that there was a disturbance in the organification of iodine.

A woman (59-year old, with obesity, 109 kg) used a treatment with resorcinol for 7 years in the last 10 years, with a dose of 4 g/d in the last 2 years (37 mg/kg/d). She experienced some clinical signs of hypothyroidism for 8 months (recent weight increase, dysesthesia in extremities, muscle cramps, hoarse voice, loss of hearing). **Diffuse hypertrophy of thyroid was observed. Biological data were in support of hypothyroidism: elevated uptake of stable iodine (9.7 µg/h); moderately elevated uptake of radioactive iodine (51% after 2h, 47% after 48h).** Administration of TSH at 48h did not impact the radioactive uptake. *Per os* administration of perchlorate at 72 and 96 h progressively decreased the radioactivity in the thyroid**. T4 level was low** (2.5 µg/100 mL; normal value 5.4 to 13 µg/100 mL). Two months after nearly complete cessation of treatment with resorcinol, the volume of goitre was reduced and clinical signs of hypothyroidy stopped. After 3 weeks, the uptake by thyroid of stable iodine was increased to 14 µg/h and plasmatic iodide level was 1.37 µg/100 mL. T4 level was elevated (16.4 µg/100 mL). After 1 month, T4 level was in the normal range (10 µg/100 mL). In addition to a disturbance in the organification of iodine, an effect on coupling of iodotyrosine was also suspected due to the slow and progressive decrease in radioactivity in thyroid after perchlorate administration (at diagnosis). It was hypothesised by the authors that resorcinol would only induce important thyroid disturbances in cases where there is an underlying thyroiditis.

Katin *et al.* (1977) report the case of a man (70-year old) who had renal failure secondary to diabetic glomerulosclerosis with other complications. The patient was in chronic hemodialysis (4 years) due to renal failure and was taking several medications. Dermal application for ca. 3 months of Lanacane® ointment for pruritis containing 2 % resorcinol and 3% benzocaine in an emollient vanishing-cream base (up to 7.5 g ointment per day) (equivalent to up to 150 mg/d) is reported. The patient was admitted to hospital because of recent onset of an **organic psychosis, weakness, lethargy and increased hoarseness. Thyroid gland was equivocally enlarged at palpation**. The patient had dry and coarse skin containing multiple senile keratosis. His skin was reported to be intact. The patient had **no detectable T3, low T4, low free thyroxine index and elevated TSH levels**. After stopping the use of ointment and commencing treatment with levothyroxine (synthetic form of T4) for 2 weeks, thyroid 24-h uptake was slightly elevated (43.2%). The thyroid was normal in size but showed isotopic localisation in the pyramidal lobe at scan. Patient's mild paranoia and confusion cleared, hoarseness improved and strength increased. After 6 months, T4 level was in the normal range. Levothyroxine treatment was stopped. T4 and TSH levels as well as 24-h thyroid uptake (28%) were normal after two weeks. T4 and TSH levels were still normal after two months.

During the public consultation of the present report, further references of human case reports were quoted in the comments (as well as in Welsch, 2008b) as case reports reporting goitres:

- Boeck, 1915
- Klem, 1930
- Strakosch, 1943

It was however not possible to further analyse this information as publications were not available.

Volunteer study

One study investigated thyroid function following dermal exposure of human volunteers to resorcinol (Yeung *et al.* 1983). Four healthy males with intact skin (aged 18 +) participated in the study. Three were allocated to the treatment group and one served as a control (exposed to vehicle). 20 ml of 2 % resorcinol in hydroalcoholic vehicle was applied twice daily, 6 days/week for 4 weeks to the face, shoulders, upper chest and upper back of the exposed subjects. The daily dose was 12 mg/kg/d (0.30 mg/cm²). 24-hour urine specimens were collected 2 and 4 weeks after initiation of treatment. Blood chemistries (SMAC 24) and thyroid functions (T3, T4, T7[10] and TSH) were measured from blood samples drawn at weeks 1, 2, 3, and 4 after the initiation of treatment (time not stated). No effect on thyroid hormones or blood chemistry were observed. All values were within normal range. However, while the normal range for TH concentrations is wide on a population level, individual levels are regulated in a narrow range (Gilbert *et al.,* 2012) and what is a normal range of TSH is under debate (Biondi, 2013). Measured values are not presented in the Yeung (1983) publication so that modifications of individual levels cannot be appreciated. No detectable levels of free resorcinol or its conjugates were measured in plasma. Conjugates but no free resorcinol were detected at week 2 in urine (see section 4.3 for further details on absorption data in this study). The absence of effect on thyroid function may reflect a low systemic exposure to resorcinol on intact skin in particular in a hydroalcoholic vehicle. Compensatory capacities such as recycling of iodine, long half-life of TH, storage by binding to plasmatic proteins, may also transiently delay the induction of effects.

Occupational studies

Data investigating the thyroid function of workers are available from two plants using resorcinol.

Workers from a plant producing resorcinol by sulphonation of benzene and also producing β-resorcylic acid, resorcinol–formaldehyde resins, sulphites, and sulphates were examined in 1978 (TOMA, 1978; Flickinger, 1976), 1980 (TOMA, 1981) and 1984 (Bauer, 1985). Reports are not available in the literature but are reported here based on their description in the WHO assessment (2006). Resorcinol 8-h TWA values are available from personal and area measurements (20 samples) for some areas of the plant. Concentrations are in a range of 0.6–66 mg/m^3. The distribution is: grinders 2–45 mg/m^3 (4 personal samples) and 2–66 mg/m^3 (4 area samples); flaker operators 0.6–2 mg/m^3 (4 personal samples) and 1–53 mg/m^3 (4 area samples); operators making pharmaceutical-grade resorcinol 0.7–2 mg/m^3 (4 personal samples). Workers were exposed primarily to resorcinol, but the exposure to other agents was not measured.

In 1978, medical examinations, chest X-rays, pulmonary function, haematology, and clinical chemistry were performed with 281 of 329 persons actively employed at the production plant. About 60% were under 40 years of age, and about 50% had worked at this plant for at least 10 years. Data concerning the different job categories are not provided. The prevalence of medical findings possibly consistent with subclinical hypothyroidism (low T4 and/or high TSH) was 5/280 (1.8%). The prevalence of possible goitre was 2/280 (0.7%). One person showed a palpable thyroid with normal T4 and TSH values (TOMA, 1978).

In 1980, the same medical examinations and thyroid assessments were performed with 247 of 387 presumably active plant workers (214 men and 33 women). About 60% were

[10] T7 or the Free Thyroxine Index (FTI) is obtained by multiplying the total T4 with T3 uptake. T3 uptake is a measure of the unbound throxine binding globulins in the blood (unsaturated with thyroid hormone). T7 is considered to be a more reliable indicator of thyroid status in the presence of other factors that may affect plasma protein binding (e.g. drugs) (Occupational medicine, 2001).

under 40 years of age, and 153 of these subjects were tested for total T4 and TSH. Five of 153 (3.3%) showed signs of clinical/subclinical hypothyroidism, but in 3 of these 5 cases, other reasons, such as treatment with radioiodine, were given as causes for the thyroid abnormalities (TOMA, 1981).

A personal communication (Bauer, 1985) quoted in WHO 2006 reports results from a follow-up of this plant performed in 1984 that includes 192 of 312 active workers. In 188 subjects (175 men, 13 women) with a mean age of 37 years, laboratory tests including medical examination, were performed. No abnormal thyroid glands or changes in T4 values were found in any of the subjects when compared with normal values.

The lack of comparison groups, exposure data and missing current and historical control data are major limitations pointed out in this series of studies.

In a textile plant where resorcinol and thiourea were used, four cases of overt hypothyroidism occurred over a period of 6 years and a follow-up study was conducted to investigate thyroid function of workers (Roberts *et al*., 1990). Clotted blood samples were collected once in January/February (n=181) or in September (n=56) from a total of 237 volunteers (189 men and 48 women). TSH, antimicrosomal (antiMS) and antithyroglobulin (antiTG) antibodies were measured. Age, sex, job title, job, department, occupational history, length of employment, history of thyroid disease and use of medication were collected by a questionnaire administered by a senior medical advisor and the company's occupational health nurse. Resorcinol and thiourea were used in the finishing department. Typical concentration at the inlet of the LEV was 5 µg/m^3 for thiourea and less than 20 µg/m^3 for resorcinol. No thiourea or resorcinol was detected in the company water supply. In the follow-up study, 15 cases of thyroid function disturbance were identified:

- 3 workers with mild hypothyroidism (defined as raised TSH and minor non-specific symptoms), including one case of mild clinical hypothyroidism (male, 53-year old, maintenance manager in daily contact with chemicals)
- 1 worker asymptomatic but with raised TSH
- 8 workers with raised circulating thyroid antibodies

Most of the cases were employed in non-process areas. However, seven of them worked in the laboratory and offices adjacent to ventilation outlets of exhaust fumes (exposure not confirmed).

In this study, concomitant use of known antithyroid agent thiourea and the absence of characterisation of the exposure to resorcinol precludes any conclusion regarding a possible resorcinol antithyroid effect.

Two other studies in the literature looked at the incidence of thyroid cancer in workers including textile workers but exposure to resorcinol was not specifically examined and these studies are not further presented (Lope *et al*., 2009; Wong *et al*., 2006).

Overall, methodological limitations prevent reaching any conclusion from the occupational studies.

<u>Environmental study</u>

Surveys were conducted on 9-14 year-old school children from 41 localities (72 to 240 in each town with equal proportion of sex and age) in western Columbia to examine the presence of naturally-occurring goitrogens contaminating water (Gaitan *et al.*, 1978; Gaitan, 1983). In these surveys, thyroid size was graded and measured according to WHO criteria. Localities received a uniform iodine supplementation in salt (50-75 mg/kg) for the last 10-20 years. Urinary samples collected from 20% of the children were examined to determine iodine excretion. **Marked differences in goitre prevalence from different localities in the Subundoy valley** (e.g. 9.6% in Santiago and 41.7% in San Antonio)

were observed even though they had similar iodine intakes, socio-economic and ethnic backgrounds.

A study was conducted in the localities of Candelaria and Zarzal since 1959 (both supplied by wells, same ethnic and socio-economic characteristics, 80 km apart). Incidence of goitre decreased significantly after iodisation in Candelaria but remained around 30% after 15 years (initial incidence >80%). At the same time, incidence of goitre in Zarzal decreased from 15.6% to 9%. In 1964 in the urban area of Candelaria, the goitre incidence was 11% in the southern part with a water supply from well B and 23% in the northern part with water supply from well A. A few years later, water from the two wells was combined in a single tank and used for the supply of the whole town. Within 12 months, goitre prevalence rose to 31% in the south and stayed approximately stable to 26% in the north.

In June 1974, 6 months after the closure of well A, the prevalence of goitre in Candelaria decreased to 8%. Well A was then reinstalled and 5 months later the prevalence of goitre again reached 31.9%. The levels of iodine intake did not explain the significant changes in goitre prevalence. **The authors concluded unequivocally on the existence of goitrogenic factors in the water** (Gaitan *et al.*, 1978).

In other localities, Gaitan (1983) reported that goitre prevalence was significantly decreased ($p<0.01$) 8 months after a modern water treatment plant (using flocculation, sedimentation, filtration and chlorination) became operational.

Geological composition of watersheds in 37 localities and bacterial contamination of water supplies in 34 localities was determined by Gaitan (1983). A multiple regression model applied to data from 16 localities found that the presence of sedimentary rocks rich in organic matters was the best indicator for disease. Presence of K. pneumoniae was associated with lower goitre prevalence and total bacterial concentration with higher prevalence. These 3 factors together accounted for 78% of the variation in goitre prevalence. Geological variables remain significantly related to goitre prevalence after adjusting for nutritional variables.

In Gaitan *et al.* (1987) (abstract with limited information), *in vivo* and *in vitro* studies for antithyroid activities were conducted on water or activated carbon extracts from Well A and Well B and demonstrated that water from Well A had an antithyroid activity. Ultrafiltration of the goitrogenic water extracts (GWE) revealed aquatic **humic substances (humic and fulvic acids) and GC/MS analysis identified over 30 organic compounds, including resorcinol**. In contrast, only 4 compounds, not including resorcinol, were identified from Well B. Fulvic and humic acids inhibit thyroid peroxidase (TPO) *in vitro* with 15% of the potency of propylthiouracil. Resorcinol has an antithyroid activity in two different rat strains. *In vitro,* resorcinol has a 15-30 times more potent effect than the antithyroid drugs methimazole and propylthiouracil on TPO inhibition, thyroid ^{125}I-uptake and thyroid hormone synthesis. No further information was provided but these data may partly correspond to the data reported in Cooksey *et al*. (1985) who investigated the antithyroid effect of eight degradation products of humic acids including resorcinol (see section 4.4.2.5).

These studies demonstrate an elevation of the prevalence of goitre in school children in relation to drinking water from a specific well containing goitrogenic vegetal extracts. Resorcinol is identified by the authors as the goitrogenic factor in water. From the limited information in this abstract, several degradation products of humic acids present in the extracts were reported to have an anti-thyroid effect including resorcinol. A cumulative effect with other thyroid disrupting substances present in the water is therefore possible.

Conclusion on human data

Goitre and clinical symptoms consistent with severe clinical hypothyroidism were reported in a total of 10 medical cases of patients exposed to resorcinol (see summary of cases in Table 7).

In all cases, enlargement of the thyroid was observed. Clinical symptoms generally included weakness/fatigue, hoarse voice, dry skin, myxoedema of the face, sensitivity to cold, dysphagia and/or dyspnea due to goitre. Depression, psychosis, loss of hearing and impairment of memory are also described (Bull & Fraser, 1950; Katin, 1977). These symptoms are characteristics of severe clinical hypothyroidism (Roberts *et al*., 2004). In seven cases, some biological parameters were measured and indicate:

- A decrease in thyroid hormones : low PBI in 3 cases (Bull & Fraser, 1950; Hobson, 1951) and low T4 and/or T3 in 2 cases (Berthezene *et al*., 1973; Katin *et al*., 1977),
- An elevated thyroid uptake of radioactive iodine in 3 cases (Bull & Fraser, 1950; Berthezene *et al*., 1973). Low urinary excretion of radioactive iodine (3 cases in Bull & Fraser 1950) was also interpreted as a sign of avid uptake by the thyroid,
- A high TSH level in one case (Katin *et al*., 1977).

Histologic examinations were available in 3 cases (Bull & Fraser, 1950) and report hyperplasia with small to large follicles, generally depleted of colloid or during the recovery phase, with peripheral vacuolation.

All these parameters are consistent with a blockage of hormone synthesis in the thyroid. Reduction of thyroid hormone plasmatic concentration leads to a reduced negative feedback on the hypothalamic-pituitary-thyroid axis, thereby resulting in increased TSH secretion. However, this mechanism is ineffective in maintaining normal thyroid hormone secretion and results in an accelerated cell dynamic of the thyroid gland, hyperplasia and goitre formation that is reported in the 10 cases. **Hypothyroidism is therefore considered well established in all ten cases.**

In all cases, resorcinol was dermally applied as an ointment, for treatment of varicose veins and/or skin ulcers and in one case for pruritis. No case was reported after 1977, which may be explained by the termination of use or reduction of concentration of resorcinol in ointments on the market. During the public consultation of the present report, Sumitomo Chemicals (Japan) has commented that topical application of ointment containing resorcinol at a maximal concentration of 5% (typically 2-3%) is still prescribed for the treatment of acne, seborrheic dermatitis, eczema, psoriasis, and other skin disorders. The extent of this use is however not known. In CICADS (WHO, 2006) it is stated that, "Resorcinol is used in pharmaceutical preparations for the topical treatment of skin conditions such as acne, seborrheic dermatitis, eczema, psoriasis, corns, and warts. Resorcinol is usually present in anti-acne preparations at a maximum concentration of 2%. The concentration of resorcinol can be much higher in peels, in some cases around 50%." A report from the Danish Ministry of the Environment (Environmental Project Nr. 942, 2004) states that "Resorcinol is found in one pharmaceutical product in Denmark, i.e. in eye drops that can be bought without prescription. Some years ago resorcinol was also found in a number of ointments against diseases like psoriasis and acne, but such products are no longer marketed in Denmark." In France, only two medicines currently contain resorcinol as an active substance with a maximum concentration of 0.03%[11].
The composition of Lanacane© that used to contain 2% resorcinol as quoted by Katin *et al*. (1977), does not include resorcinol anymore (https://www.medicines.org.uk/emc/product/5952/smpc, accessed on 5 May 2020).

[11] Source: ANSM database http://agence-prd.ansm.sante.fr/php/ecodex/index.php

Although systemic exposure to resorcinol was confirmed in only one case (presence of resorcinol conjugates in urine in Thomas & Gisburn 1961), a regular external long-term exposure to resorcinol was established in all cases. Known durations of exposure ranged from 3 months to 13 years and estimated daily doses from 2 to 140 mg/kg/d.
The composition of the ointment is described in three of the case reports (case 2 of Bull & Fraser 1950, case described by Hart & Maclagan 1951 and case described by Thomas & Gisburn 1961). Lanolin, glycerin, paraffin and in one case zinc oxide were the other ingredients. Cresol was also reported in a very small amount of 0.02% in Hart & Maclagan (1951). In addition, in Katin *et al*. (1977) the full composition is not available but benzocaine (3%) was mentioned as the other active substances of the ointment together with resorcinol. No thyroid effect is reported as a side effect of benzocaine despite large use as a local anaesthetic (Singh & Al Khalili, 2020). All publications have concluded on a link between the effects reported with the exposure to resorcinol. This conclusion is also strongly supported by the effect of resorcinol on TPO that has been established *in vitro*, providing strong biological plausibility (see section 4.6).

In nine of the patients, resorcinol was applied on ulcerated skin secondary to varicose veins and application on damaged skin probably contributed to an important systemic absorption of resorcinol due to the absent or limited skin barrier and to an potential altered skin metabolisation. In one patient exposed to 2 mg/kg/d for 3 months (Katin *et al*., 1977) the skin was reported as intact. In this patient, renal failure may however have altered the elimination of resorcinol metabolites and disturbed the rapid metabolism of resorcinol. These conditions are expected to have an impact on the systemic availability of resorcinol as further discussed in section 6.3.2.1 in relation to the level of concern. However, a skin absorption potential exists and the latter human case support that damaged skin is not a requirement to induce such effects. These data are considered relevant for the assessment of the intrinsic properties of resorcinol in general and of its endocrine disrupting properties in particular.

Medical history in relation to the case was described in all case reports. In total, three of the patients suffered from severe pathologies (cardiac insufficiency, obesity, renal failure and diabetes) and took drugs for these pathologies. This may have influenced or contributed to alteration of the thyroid function of the patients. However, two patients were described as in general good health. No pre-existing pathology was described in addition to ulcered varicose veins for four additional patients. None of the patients were described to have an underlying thyroid dysfunction. Considering the effects observed after the treatment, it is likely that such a pre-existing pathology would have been reported. It cannot be excluded that a subclinical and/or undetected thyroid condition was present for some cases but this pathology is quite frequent in the human population (3 to 10% reported in Schuebel *et al*., 2017) and it would not make the human cases irrelevant.

Finally, the effects described in human case reports are fully consistent with the action of resorcinol on TPO inhibition that is well established *in vitro* (see section 4.5.1). Biological plausibility therefore supports that resorcinol is the causal agent inducing hypothyroidism and goiter, as further discussed in section 4.6.

The human cases are considered to provide evidence of an adverse effect in intact organisms in the meaning of the WHO/IPCS (2002) definition of an ED. Reference to intact organisms is intended to exclude in the demonstration of adverse effects, data from experimental models artificially altered that do not reflect hormonal regulation under realistic physiological conditions. OECD TG 150 (2018) states that "*the term "intact organism" is understood to mean that the effect would occur in vivo, either observable in a test animal system, epidemiologically or clinically*". In some cases the subjects/persons were ill and in most cases the patients had broken skin/skin lesions. But, as clinically-observed cases, they are intact organisms in the sense given in the above mentioned definition. They have not been modified to be more sensitive to endocrine disruption and can therefore not be considered as non-intact organisms.

The evidence of a causal relationship between resorcinol and the hypothyroidism comes in particular from the regression of the goitre and symptoms after cessation of exposure to resorcinol in eight of the ten cases. In the two other cases, death of the patients (due to heart failure or extensive thrombosis) did not allow further investigations. It shows that even though resorcinol may not be the only factor responsible for the hypothyroidism, it is the determining one.

A volunteer study (Yeung *et al*., 1983) and studies conducted in occupational settings (TOMA, 1978; TOMA, 1981; Roberts *et al*., 1990) do not allow to conclude on the absence of thyroid effects related to exposure to resorcinol because of methodological limits.

Resorcinol present in drinking water was suspected to be the main substance explaining the locally elevated incidence of goiters in children in western Columbia (Gaitan *et al*., 1978 and 1983).

Table 7: Summary of medical cases

HUMAN CASE STUDIES		
Background	**Findings**	**Reference**
Case 1: woman with cardiac insufficiency with ulceration of the skin; resorcinol dose and duration not known **Case 2:** woman in good general health with ulceration of the skin; approx. 26-52 mg/kg/d resorcinol for 2 years **Case 3:** woman in good general health with ulceration of the skin; approx. 37 mg/kg/d resorcinol for 3 years.	**Case 1:** hypothyroidism observed (goitre, clinical signs, histology) **Case 2:** hypothyroidism observed (goitre, clinical signs, histology, low serum-bound iodine level). Reversal after cessation of application of resorcinol. **Case 3:** hypothyroidism observed (goitre, clinical signs, histology, low serum-bound iodine level). Reversal after cessation of application of resorcinol.	Bull & Fraser *et al*. 1950
Woman with ulceration of the skin; resorcinol ointment (4%) for 12 years (dose not possible to estimate)	Hypothyroidism observed for 5 years (goitre, clinical signs, high iodine uptake). Reversal after cessation of application of resorcinol.	Hart & Maclagan 1951
Man with ulceration of the skin; resorcinol ointment (4% then 12 %) for 9 months (dose not possible to estimate).	Hypothyroidism observed (goitre, clinical signs, low protein-bound iodine level). Reversal after cessation of application of resorcinol.	Hobson 1951
Woman with ulceration of the skin; resorcinol ointment (12.5%) for 13 years; up to approx. 875 mg/d in the 2 last years (17.5 mg/kg/d assuming a 50 kg BW)	Hypothyroidism observed (goitre, clinical signs). Clinical symptoms relieved by treatment with thyroid tablets. Resorcinol conjugates in urine.	Thomas & Gisburn, 1961
Woman with ulceration of the skin; resorcinol ointment (2 %) for one year (dose not possible to estimate).	Hypothyroidism observed after a few months (goitre, clinical signs, high iodine uptake).	Guinet *et al.* 1967 (In French)

HUMAN CASE STUDIES Background	Findings	Reference
	Reversal after cessation of application of resorcinol and treatment to liothyroxine.	
Case 1 : woman with pre-diabetic condition, 96 kg and with ulceration of the skin; approx. 7 g/day resorcinol for 5 years (7373 mg/kg/d) **Case 2**: woman with obesity (109 kg) and with ulceration of the skin; resorcinol for 7 years with 4 g/d in the last 2 years (337 mg/kg/d)	**Case 1:** hypothyroidism observed for 1 year (goitre, clinical signs, high iodine uptake). Reduction one month after cessation of application of resorcinol. **Case 2:** hypothyroidism observed for 8 months (goitre, clinical signs, high iodine uptake). Reversal two months after cessation of application of resorcinol.	Berthezene *et al*. 1973 (In French)
Man with renal failure secondary to diabetic glomerulosclerosis; intact skin. Approx. 150 mg/d resorcinol for 3 months (2 mg/kg/d assuming a 70 kg BW)	Hypothyroidism observed (goitre, clinical signs, low T3/T4, high TSH). Reversal 6 months after cessation of application of resorcinol and treatment with levothyroxine.	Katin *et al*. 1977

4.4.2. Experimental data (*in vivo*)

4.4.2.1. Single exposure (all routes of exposure)

Some studies investigated the effect of resorcinol on thyroid function after a single exposure. Data are summarised in Table 8 below.

Doniach & Fraser (1950) (ToxRtool score 3) exposed rats (hooded Lister strain, mostly females) to graded doses of resorcinol injected subcutaneously or to resorcinol in drinking water as a 2% solution. Radioactive potassium iodide was injected intraperitoneally 10-30 min after exposure to resorcinol. Animals were sacrified 1, 1.5 or 2 h after iodide injection and radioactivity determined in thyroid tissues. No information is available on dietary iodine intake of animals. **Subcutaneous injection of 5 mg/kg resorcinol or more reduced iodine uptake to 11-17% of controls** (comparable to maximal reduction obtained with methyl thiouracil – MTU). The result did not differ significantly when two separate subcutaneous doses were administered. When resorcinol was administered in drinking water (dose not known), there was an indication in some animals of a **smaller but definite antithyroid effect on iodine uptake.** Antithyroid effect observed with resorcinol was enhanced by the additional injection of thiocyanate, an inhibitor of NIS, but not of MTU, a TPO inhibitor. The authors concluded that thiocyanate prevents the uptake of some of the iodide ion which has been concentrated in the thyroid despite the presence of resorcinol.

Arnott & Doniach (1952) (ToxRtool score 2) performed a series of experiments to determine the antithyroid activity of resorcinol and other phenols. All experiments were performed in albino rats (n=4 /group unless otherwise specified). No information is available on dietary iodine intake of animals. Ten to twenty minutes after a single subcutaneous injection of test substance or vehicle, animals received an intraperitoneal injection of radioactive iodine and were killed 2 h later and ^{131}I uptake in their thyroid

measured. **A dose of 70 mg/kg of resorcinol in water induced a marked anti-thyroid activity as thyroid ^{131}I uptake was decreased to 11% of controls** ($p<0.001$). Signs of toxicity were absent or minimal. A similar decrease of 14% of controls ($p<0.001$) was reproduced at the same dose of 70 mg/kg (number of test animals not specified). Doses of 42, 70, 90 or 180 mg/kg of resorcinol were tested and the **thyroid ^{131}I uptake was respectively 40% (p=0.05-0.02), 24% (mean of 11 experiments, range: 11-45%), 23% (p=0.01-0.001, 2 rats) and 14% (mean of 9 experiments, range: 8-23%)** of the controls. The anti-thyroid effect was not dose related which increases the uncertainty on a safe-dose level.

Doniach & Logothetopoulos (1953) (ToxRtool score 2) carried out a series of experiments in rats (see sections 4.4.2.2 and 4.4.2.3 for results of repeated exposure). No information is available on dietary iodine intake of animals in these experiments.

Rats (n=5/group) were exposed to a single subcutaneous injection of 55 mg/kg of resorcinol in water and were administered an injection of radioactive iodine 3 h later. Animals were killed 2 h after injection of radioactive iodine and thyroid uptake was measured. No significant difference was observed in the ^{131}I thyroid uptake (90.4% of controls).

In a poorly reported study, Berthezene *et al.* (1979) (ToxRtool score 3) exposed rats deficient in iodine (strain, sex and number not specified) to 24.5 mg resorcinol including radioactive isotope by the intravenous (iv) route. No information is available on dietary iodine intake of animals. **A decrease in thyroid iodine uptake was observed 2h 30min after administration (61.4±0.8% vs 71.2±0.6% in control, p<0.001).** Radioactive iodotyrosine/iothyronine ratio was increased. Radioactive thyroid iodide and MIT/DIT ratio were not modified.

Table 8 : Overview of single dose toxicity study

SINGLE DOSE TOXICITY				
Study design	**ToxRtool score**	**Effects on thyroid**	**Other results**	**Reference**
Rats (hooded Lister strain) (ND on I content in diet) Subcutaneous injection of resorcinol in water or administration in drinking water as a 2% solution Doses: approx. 1 to 300 mg/kg given in one or two administration	ToxRtool score 3 Minor limitations: ▪ experimental conditions poorly described, Major limitations: ▪ low numbers of animals, ▪ limited reporting of results, ▪ absence of statistical analysis. Used in a WoE approach as supporting study	Subcut. injection ≥ 5 mg/kg: 11-17% reduction of iodine uptake (comparable to MTU). Drinking water: indication in some animals of a smaller but definite antithyroid effect.	Doses of approx. 250 mg/kg induced severe tremors for the first half-hour. No lethality.	Doniach & Fraser (1950)
Albino rats (n=4 /group unless otherwise specified) (ND on I content in diet) Single subcutaneous administration of resorcinol in water (other compounds	ToxRtool score 2 Minor limitations : ▪ absence of information on the sex of animals, housing and feeding conditions ▪ low number of animals	^{131}I uptake compared to controls: <u>42 mg/kg:</u> 40% (p=0.05-0.02) <u>70 mg/kg:</u> 1st exp: 11% (p<0.001) 2nd exp: 11% (p<0.001)	Toxic effects reported for most of the compounds tested at 180 mg/kg: intense shivering after administration followed by lethargy Toxic effects absent or minimal	Arnott & Doniach (1952)

SINGLE DOSE TOXICITY				
Study design	**ToxRtool score**	**Effects on thyroid**	**Other results**	**Reference**
tested) Doses: 42 to 180 mg/kg resorcinol		All exp: 24% (mean of 11 exp., range 11-45%) 90 mg/kg: 23% (p=0.01-0.001, 2 rats) 180 mg/kg: 14% (mean of 9 experiments, range: 8-23%)	at 70 mg/kg (information not given specifically for resorcinol)	
Rats (n=5/group) (ND on I content in diet) Single subcutaneous injection of 55 mg/kg of resorcinol in water	ToxRtool 2 Minor limitations: ▪ experimental conditions poorly described (absence of information on purity of test substance, housing and feeding conditions), ▪ low numbers of animals.	No significant difference observed in the ^{131}I thyroid uptake (90.4% of controls).	No information	Doniach & Logothetopoulos, 1953
Rats deficient in iodine (strain, sex and number not specified) (ND on I content in diet) Single intravenous injection of 24.5 mg resorcinol	ToxRtool score 3 Major limitations: ▪ experimental conditions poorly described (absence of information on purity of test substance, number of animals, housing and feeding conditions, details of experimental protocol etc), ▪ limited reporting of results. Used in a WoE approach as supporting study	Thyroid iodine uptake decreased 2h30 after administration : 61.4±0.8% vs 71.2±0.6% in control, p<0.001 Increased Radioactive iodotyrosine/iothyronine ratio Radioactive thyroid iodide and MIT/DIT ratio not modified.	No information	Berthezene *et al*. 1979

ND: no data; I: iodine

4.4.2.2. Subcutaneous route – repeated exposure

Data are summarised in Table 10 below.

Klein *et al*. (1950) (ToxRtool score 3) exposed subcutaneously seven rabbits (6 males, 1 female) to 50 mg/kg/d resorcinol in 0.9% saline solution for 4 days and 75 mg/kg/d for 15 days. No information is available on dietary iodine intake of animals. The animals lost about 5% of their weight at the end of the treatment. **The thyroid of these animals showed no sign of enlargement and no histological modifications.**

Seven males were exposed to the same protocol but together with administration of MTU (100 mg/kg/d 6 day per week) 6 weeks before and during exposure to resorcinol.

Thyroids were enlarged and showed histologic modification consistent with previous observations further to exposure to MTU but these data are not presented in the publications.

Cheymol *et al.* (1951) (ToxRtool score 3) exposed subcutaneously 12 male Wistar rats to 50 mg/kg resorcinol, pyrocatecol or hydroquinol in aqueous solution every two days for one month. Nine animals served as negative controls. No information is available on dietary iodine intake of animals. Thyroids were weighed and examined microscopically. Tremors were observed during the hour following the injection. **Relative thyroid weight was 13.8 mg/100 g BW in the resorcinol group compared to 11.5 mg/100 g BW in the control group (no statistical analysis provided in the publication). Histologic examination showed a slight reduction in colloid with resorcinol.**

Doniach & Logothetopoulos (1953) (ToxRtool score 2) carried out a series of experiments in rats (see section 4.4.2.1 for results of single exposure and 4.4.2.3 for results on repeated dermal exposure). In these experiments, no information is available on dietary iodine intake of animals.

Four rats were subcutaneously exposed twice daily to resorcinol in oil (estimated dose of 308 mg/kg/d) for 10, 31, 47 or 69 days respectively, and 3 control rats were exposed to oil only for 47, 69 or 69 days, respectively. Thyroids of controls showed normal histology and weight (16, 18 and 13 mg, respectively)). The thyroids of rats exposed to resorcinol for 10 and 31 days appeared macroscopically normal but were not further weighed or examined. **The thyroid of the rat exposed to resorcinol for 47 days weighed 32 mg and showed hyperaemia, gross cellular hyperplasia and widespread depletion of colloid. The thyroid of the rat exposed to resorcinol for 69 days weighed 25 mg and showed moderate hyperplasia. Therefore, some signs of hypothyroidy were observed from 47 days of exposure but the severity was not time-dependent**. The interpretation is however limited by the very low number of animals and the potential individual variations in sensitivity.

Subcutaneous injection of an estimated dose of 990 mg/kg/d of warm resorcinol in beeswax-arachis-oil mixture induceed convulsions and killed the rats after a few days of treatment.

Samuel (1955) (ToxRtool score 3) reported a series of experiments performed with Wistar rats (albino) to investigate the effect of resorcinol on thyroid function. Animals were fed with Standard Purina fox chow diet (no specific information on iodine content). Thyroid glands were weighed and thyroid, pituitary, adrenal glands, liver and heart were examined microscopically after sacrifice of the animals. The first experiment was performed by dermal route and reported below in section 4.4.2.3.

In the second experiment, female rats were subcutaneously exposed twice per day to resorcinol in peanut oil at various doses and for various durations. In two animals exposed to 308 mg/kg/d (dose estimated from information in the publication) for 21 or 38 days, **thyroid weight was 17 and 16 mg, respectively, vs 13 and 14 mg in the two controls exposed to peanut oil.** Microscopically, mild or early changes in the form of thinner colloid and somewhat taller epithelial cells were observed. In three animals exposed to 396 mg/kg/d (dose estimated from information in the publication) for 52, 78 or 39 days the thyroid weight was 22, 28 and 28 mg, respectively. **Thyroids showed marked hyperplasia, acini were devoid of colloid and varied in size and shape**. The lining cells were tall cuboidal and vascularity was increased. Some evidence of an increase in pale basophil cells was observed in the anterior lobe of the pituitary in two animals. Focal necrosis and cystic degeneration were observed near the site of multiple injections.

In the third experiment, five female rats were subcutaneously exposed twice per day to resorcinol with 2% beeswax in peanut oil (total dose estimated as 792 mg/kg/d) for 21 to 79 days. Thyroid weights are reported below in Table 9. In the 3 animals with a two-fold increase in thyroid weight, histologic changes similar to those previously described in experiment 2 were observed although the epithelial cells were tall columnar only in patchy areas and mitotic figures were few or lacking entirely. The pituitary and adrenal glands of these animals showed changes similar to experiment 1 but to a lesser degree.

Table 9: Thyroid weight in the 3rd experiment

	Controls		Resorcinol				
Duration of exposure (d)	35	79	21	29	35	49	79
Body weight at the end of experiment (g)	350	270	260	265	350	230	350
Thyroid weight (mg)	11	13	26	19	37	19	32
Relative thyroid wt (mg/g BW)	0.03	0.05	0.10	0.07	0.11	0.08	0.09

In a final experiment, co-administration of potassium iodide did not inhibit hyperplasia induced by subcutaneous administration of resorcinol (1 rat tested with resorcinol and 1 rat tested with resorcinol diacetate) for 38 or 45 days.

Table 10 : Overview of repeated dose toxicity study via subcutaneous route

	REPEATED DOSE TOXICITY, SUBCUTANEOUS ROUTE			
Study	**ToxRtool score**	**Effects on thyroid**	**Other results**	**Reference**
Rabbits (n=7/group) (ND on I content in diet) 50 mg/kg/d for 4 d or 75 mg/kg/d for 15 d (in 0.9% saline)	ToxRtool 3 Minor limitations: ▪ experimental conditions poorly described (absence of information on purity of test substance, housing and feeding conditions) Major limitations: ▪ absence of statistical analysis ▪ limited reporting of results. Used in a WoE approach as supporting study	No effect on size or histology	Loss of 5% weight	Klein *et al*. 1950
Wistar rats (n=12 males) (ND on I content in diet) 50 mg/kg/d every two days for one month (in aqueous solution)	ToxRtool 3 Minor limitations: ▪ experimental conditions poorly described (absence of information on purity of test substance, housing and feeding conditions). Major limitations: ▪ absence of statistical analysis ▪ limited reporting of results. Used in a WoE approach as supporting study	Higher thyroid weight but statistical significance not known. Slight reduction in colloid.	Tremors during the hour after the injection	Cheymol *et al*. 1951
Rats (n=4) (ND on I content in diet) 308 mg/kg/d for 10 to 69	ToxRtool 2 Minor limitations: ▪ experimental conditions poorly described (absence of information on purity of test substance, housing and feeding	Increased thyroid weight and histological findings (hyperaemia, cellular hyperplasia, depletion of colloid) from	ND	Doniach & Logothetopoulos, 1953

Study	REPEATED DOSE TOXICITY, SUBCUTANEOUS ROUTE ToxRtool score	Effects on thyroid	Other results	Reference
days (in oil)	conditions), ▪ low numbers of animals.	exposure ≥47 d		
Wistar rats (1 female/dose/duration) (Standard Purina fox Chow diet; ND on I content in diet) 308 or 396 mg/kg/d for 21 to 38 days (in peanut oil)	ToxRtool 3 Minor limitations: ▪ experimental conditions poorly described (absence of information on purity of test substance) ▪ low numbers of animals. Major limitations: ▪ expression of doses unclear, ▪ absence of statistical analysis. Used in a WoE approach as supporting study	308 mg/kg (21 to 38 days): no change in thyroid weight; thinner colloid. 396 mg/kg (39-78 days): increased thyroid weight and histological findings (hyperplasia, depletion of colloid).	Histological effects in pituitary. Focal necrosis and cystic degeneration at site of injection.	Samuel, 1955
Wistar rats (1 female/dose/duration) 792 mg/kg/d for 21 to 38 days (in 2% beeswax in peanut oil)		Increased thyroid weight and histological findings (hyperplasia, depletion of colloid) in some animals.	Changes in pituitary and adrenal glands in animals with thyroid findings.	

ND: no data; I: iodine

4.4.2.3. Dermal route – repeated exposure

Data are summarised in Table 11 below.

Doniach & Logothetopoulos (1953) (ToxRtool score 2) carried out a series of experiments in rats (see section 4.4.2.1 for results of single exposure and 4.4.2.2 for results on repeated subcutaneous exposure). No information is available on dietary iodine intake of animals in these experiments.

The shaved belly of six rats was rubbed for one hour twice daily for 3 weeks with an ointment with 12.5% resorcinol. Six rats were exposed to the ointment base only. No effect was reported on the weight of the thyroid.

The first experiment of a series was performed via the dermal route by Samuel (1955) (ToxRtool score 3) (see section 4.4.2.2 for experiments by subcutaneous route). Animals were fed with Standard Purina fox chow diet (no specific information on iodine content). The ventral surface of 14 Wistar rats (albino) was shaved. An ointment (approx. 6 g/application) containing 12.5% resorcinol was applied to 6 rats daily (10 min twice daily) for 28 days. Three rats received the same exposure regime but ventral skin was scarified before the first application. Three rats were scarified and received ointment without resorcinol. The dose of resorcinol applied in treated rats can be estimated based on information available in the publication to approximately 8 g/kg/d. All rats gained weight during the experiment. **Thyroid average weight was 3.3 times greater in treated compared to control rats. The thyroid weights were respectively 45±8.5, 45±8.3 and 14±2.3 mg in the animals treated with resorcinol, resorcinol+scarification**

and scarification only (no statistical analysis presented; SD calculated from data presented in the publication). The enlargement was diffuse and bilateral. The glands were dark red from hyperemia. The other organs appeared normal on gross examination. **Microscopic examination of thyroid revealed uniform diffuse hyperplasia in marked contrast with controls.** The acini were highly variable in size and shape and many were almost devoid of colloid. The epithelium showed proliferative activity. Microscopic changes were also observed in adrenals (large vacuolated cells with large cell nuclei in zona glomerulosa and outer part of zona fasciculate) and in pituitary (marked prominence of basophilic cells) of treated animals.

In Stenbäck & Shubik (1974) (ToxRtool score 3), female Swiss mice (n=50/group) were dermally exposed to different substances including resorcinol at a concentration of 5, 25 or 50% in acetone twice a week (approximately 1, 5 and 10 mg resorcinol per animal). Animals were fed a commercial diet (no information on iodine content). Animals exposed to resorcinol showed skin lesions with ulceration, inflammation and hyperplasia. No significant increase of tumour was observed. No other findings were reported (no detailed information provided).

A similar study was performed on New-Zealand rabbits (n=5/group, both sexes) and reported in Stenbäck (1977) (ToxRtool score 3). Resorcinol was dissolved in acetone or methanol (not further specified in the publication) and applied at 5, 10 or 50% twice a week. Animals were fed a commercial diet (no information on iodine content). No effect on survival rate, no local changes and no tumours were reported in animals exposed to resorcinol.

It is noted that the use of acetone as a vehicle in these two studies may have enhanced the evaporation of resorcinol. In addition, it is not known whether the thyroid was examined and these studies have not been further considered in the analysis.

Table 11: Overview of repeated dose toxicity study via dermal route

	REPEATED DOSE TOXICITY, DERMAL ROUTE			
Study	**ToxRtool score**	**Effects on thyroid**	**Other results**	**Reference**
Rats (n=6) (ND on I content in diet) Dermal, 12.5% resorcinol in ointment 3 wk exposure	ToxRtool 2 Minor limitations: ▪ experimental conditions poorly described (absence of information on purity of test substance, housing and feeding conditions), ▪ low numbers of animals.	No effect	ND	Doniach & Logothetopoulos, 1953
Wistar rats (n=6) (Standard Purina fox Chow diet; ND on I content in diet) Dermal, in ointment 8 g/kg/d for 28 days	ToxRtool 3 Minor limitations: ▪ experimental conditions poorly described (absence of information on purity of test substance) ▪ low numbers of animals. Major limitations: ▪ expression of doses unclear, ▪ absence of statistical analysis. Used in a WoE approach as supporting study	Increased thyroid weight and histological findings (hyperaemia, epithelial proliferation, depletion of colloid) from exposure ≥47 d	All rats gained weight. Microscopic changes in adrenals (large vacuolated cells with large cell nuclei in zona glomerulosa and outer part of zona fasciculate) and in pituitary (marked prominence of basophilic cells)	Samuel, 1955
Swiss mice (50	ToxRtool 3	No effect reported	Skin lesions with	Stenbäck &

Study	REPEATED DOSE TOXICITY, DERMAL ROUTE ToxRtool score	Effects on thyroid	Other results	Reference
females/group) (ND on I content in diet) Dermal 1, 5 or 10 mg/animal (in acetone) for life	Minor limitations: ▪ experimental conditions poorly described (absence of information on purity of test substance) Major limitations: ▪ very limited presentation of experimental results. Used in a WoE approach as supporting study	but not known whether thyroid was examined.	ulceration, inflammation and hyperplasia No effect on survival, no tumours	Shubik, 1974
New-Zealand rabbits (5/group) (ND on I content in diet) Dermal 1, 5 or 10 mg/animal (in acetone or methanol) for life	ToxRtool 3 Minor limitations: ▪ experimental conditions poorly described (absence of information on purity of test substance) Major limitations: ▪ very limited presentation of experimental results. Used in a WoE approach as supporting study	No effect reported but not known whether thyroid was examined.	No effect on survival, no local changes, no tumours	Stenbäck, 1977

ND: no data; I: iodine

4.4.2.4. Gavage – repeated exposure

Data are summarised in Table 15 at the end of the section.

Repeated-dose toxicity and carcinogenicity studies

NTP conducted preliminary 17-day studies and 90-day and 2-year studies in B6C3F[1] mice and F344/N rats by gavage (resorcinol, purity > 99%, administered in deionised water) (NTP 1992) (ToxRtool score 1). In these experiments, animals were fed with NIH-07 diet, with an iodine content of 3.37 mg/kg (as calcium iodate).

In the preliminary 17-day mice and rats study, thyroids were not weighed and not processed for microscopic examination and thyroid hormones were not measured. These studies are therefore not further detailed.

In the 90-day study, mice (n=10/sex/group) were exposed to 0, 28, 56, 112, 225 or 420 mg/kg/d. Eight mice of each sex receiving 420 mg/kg resorcinol died and surviving males had a significantly lower body weight change. The final mean body weights of dosed mice in all groups were similar to those of the control groups. Clinical signs (dyspnea, prostration, tremors) were observed in high-dose animals. A statistically significant decrease in adrenal gland relative and absolute weight was observed in males at all doses (except high dose with only 2 surviving animals). In females, relative adrenal gland weight was significantly increased at 225 mg/kg/d. Thyroids were not weighed but histopathologic examination was performed. **No chemical-related gross or microscopic lesions were observed in the thyroid or in any other organ examined**. Thyroid hormones were not measured.

In the 2-year study, mice (n=60/sex/group) were exposed to 0, 112 or 225 mg/kg/d. The

mean body weight of high-dose females was 10 to 15% lower than controls from week 85. Terminal survival was unaffected by treatment. Clinical signs (recumbency and tremors after dosing) were observed in both sexes. Survival was unaffected by treatment. **No increased incidence in neoplasms or non-neoplastic lesions was observed at any site**. Thyroids were not weighed but histopathologic examination was performed. Thyroid hormones were not measured.

In the 90-day study, rats (n=10/sex/group) were exposed to 0, 32, 65, 130, 260 or 520 mg/kg/d. All females and 8/10 males died during the four first weeks at the highest dose. The final mean body weights of dosed rats in all groups were similar to those of the control groups. Clinical signs (tremors) were observed in high-dose animals. A statistically significant increase in adrenal gland relative and absolute weight was observed in males at all doses. Absolute and relative liver weights were significantly increased in males from 130 mg/kg/d and in females from 65 mg/kg/d. Thyroids were not weighed but histopathologic examination was performed. **No chemical-related gross or microscopic lesions were observed in the thyroid or in any other organ examined**. **T3 and T4** were measured in controls and in animals dosed with 130 mg/kg/d and **no significant effect was observed** (T3: 109±6 in treated vs 107±7 µg/dL in control males, 112±4 in treated vs 118±8 µg/dL in control females; T4: 7±0 in treated vs 7±0 µg/dL in control males, 4±0 in treated vs 5±0 µg/dL in control females).

In the 2-year study, male rats (n=60/sex/group) were exposed to 0, 112 or 225 mg/kg/d and female rats to 50, 100 or 150 mg/kg/d. The mean body weight of high-dose males and females were 10 to 15% lower than controls from week 87 and 95, respectively and their survival was reduced. Clinical signs (ataxia, prostration, salivation, tremors after dosing) were observed in all treated males and in females from 100 mg/kg/d. Thyroids were not weighed but histopathologic examination was performed. **No significant histopathological findings, including tumours, were observed in the thyroid**. The incidence of thyroid C-cell adenoma was 6%, 8% and 10% in males exposed to 0, 112 or 225 mg/kg/d, respectively (no significant effect) and no carcinoma were observed. The incidence of thyroid C-cell tumours was 4%, 6%, 8% and 6% for adenomas in females exposed to 0, 50, 100 or 150 mg/kg, respectively and 4%, 2%, 0% and 2% for carcinomas (no significant effect). No statistically or biologically (above HCD) increased incidence in neoplasms or non-neoplastic lesions was observed at any other site. Thyroid hormones were not measured.

Another 90-day study was conducted in Sprague Dawley rats by gavage (Unpublished study report 2004a). The study was performed according to OECD TG 408 (ToxRtool score 1). Animals were fed with A04 C pelleted maintenance diet, with an iodine content of 0.3 mg/kg. Animals were exposed to 0, 40, 80 or 250 mg/kg/d resorcinol in purified water for 13 weeks. Six controls and high-dose animals of each sex were then kept for a 4-week treatment-free period. Thyroid were weighted and histopathological analyses performed. The levels of thyroid hormones were not determined.

No treatment-related mortality was observed. Animals of the high-dose group showed intermittent convulsive movements and excessive salivation, starting approximately between weeks 6 and 8. Body weight was transiently reduced in females between weeks 4 and 8 but no effect on body weight was observed at the end of the exposure period. At week 13, plasma levels were quantifiable only at 0.5 to 2 hours for the 80 and 250 mg/kg/d groups (LOQ=0.5 µg/mL). AUC in females was approximately 3 times higher than in males (see section 4.3 for more detail on toxicokinetic investigations). The functional observation battery (FOB) performed after week 10 revealed an increased landing foot splay in females exposed to 80 and 250 mg/kg/d. Motor activity was unaffected by treatment. **Absolute thyroid weight (see Table 12 below) was significantly decreased by 19% in high-dose females at the end of exposure period and significantly increased by 37% after 4-week without exposure**. These changes are substantial but not significant when relative weight is considered (-13% at the end of exposure and +30% after recovery). No

significant changes in any other organ weight were observed and there were no histopathological findings in any organ.

Table 12: Thyroid weights in the 90-day study in Sprague-Dawley rats

Dose (mg/kg/d)		0	40	80	250
MALES					
End of exposure	Abs.	0.02700±0.006	0.02640±0.004	0.02613±0.004	0.02300±0.005
	Rel.	0.00528±0.001	0.00555±0.001	0.00517±0.001	0.00474±0.001
After 4-wk recovery	Abs.	0.02467±0.008	-	-	0.02417±0.005
	Rel.	0.00485±0.001	-	-	0.00443±0.001
FEMALES					
End of exposure	Abs.	0.01890±0.003	0.01890±0.003	0.01860±0.004	0.01540*±0.003
	Rel.	0.00680±0.001	0.00688±0.001	0.00690±0.001	0.00589±0.001
After 4-wk recovery	Abs.	0.01633±0.003	-	-	0.02240**±0.006
	Rel.	0.00585±0.001	-	-	0.00764±0.002

Abs.: absolute weight (g); Rel.: relative weight (%); * p<0.05; ** p>0.01

Developmental toxicity studies

Pregnant Sprague-Dawley dams (n=23/group) were exposed to 40, 80 or 250 mg/kg/d resorcinol in distilled water from gestational day (GD) 6 to 15 (Unpublished study report, 1982a). The study was performed according to the guideline available at that time and similar to the current OECD TG 414, except that exposure was stopped early (GD 15) (ToxRtool score 1). Animals were fed with Ssniff R rat diet (no information on iodine content). Maternal toxicity was limited to a transient reduction of body weight gain from day 6 to 15 in high-dosed females, but this reduction is not statistically significant. No effect was observed on body-weight gain over the whole gestation. No clinical signs and no maternal mortality were observed. No effect was observed on the number of corpora lutea, implantations, foetal viability, foetal weight and foetal malformations. An increased incidence of skeletal variations (see Table 13 below) was observed (2%, 7.7%, 8.5% and 10.5% in groups exposed to 0, 40, 80 or 250 mg/kg/d) and consisted of **parietal incompletely ossified, interparietal incompletely ossified, splitting of ossification centres, single extra ribs and extra pair of ribs**. They were observed in foetuses of normal weight in the study. These variations cannot be linked to weight loss and to excessive toxicity. No historical control data were provided.

Table 13: Total and individual foetal incidence of skeletal variations observed in Sprague-Dawley rats (unpublished study report, 1982a)

FOETAL SKELETAL VARIATIONS				
	Resorcinol dose (mg/kg bw/day)			
	0	**40**	**80**	**250**
Foetuses evaluated (number)	98	91	117	142
Incomplete ossification of PARIETALS Foetal incidence: number (%) Litter incidence: number	 2 (2%) 2	 5 (5.5%) 4	 4 (3.4%) 3	 7 (4.9%) 3
Incomplete ossification of INTERPARIETAL Foetal incidence: number (%) Litter incidence: number	 0 0	 1 (1.1%) 1	 2 (1.7%) 2	 5 (3.5%) 3
Splitting of ossification centres Foetal incidence: number (%) Litter incidence: number	 0 0	 1 (1.1%) 1	 1 (0.8%) 1	 3 (2.1%) 3
Single extra rib Foetal incidence: number (%)	 0	 0	 2 (1.7%)	 4 (2.8%)

FOETAL SKELETAL VARIATIONS				
	Resorcinol dose (mg/kg bw/day)			
	0	40	80	250
Litter incidence: number	0	0	2	3
Extra pair of ribs Foetal incidence: number (%) Litter incidence: number	 0 0	 0 0	 0 0	 2 (1.4%) 2
Total skeletal variations Foetal incidence: number (%) Litter incidence: number	 2 (2.0%) 2	 7 (7.7%) 5	 10 (8.5%) 6	 15 (10.5%) 8

* p < 0.05 and ** p < 0.01 (Fishers exact test)

Similarly, in DiNardo *et al*. (1985), pregnant Sprague-Dawley dams (n=13/group) were exposed to 0, 125, 250 or 500 mg/kg/d resorcinol in propylene glycol by gavage from GD 6 to 15 (ToxRtool score 3). Animals were fed with Purina Laboratory Rodent Chow. An iodine content of 1 mg/kg is reported for this diet from public source. Maternal weight gain was reduced during GD 6-16 and 16-20 at the highest dose but it did not reach statistical significance. It is not known whether it is related to a lower foetal weight or a lower weight of dams' body. The study reports that resorcinol does not induce any teratogenic effects after visceral and skeletal examinations and assessment of foetal viability and body weights but no detailed information was presented (no numerical data).

In a developmental toxicity study compliant with OECD TG 414 (ToxRtool score 1), pregnant Sprague-Dawley rats (n=24/group) were exposed to 0, 40, 80 or 250 mg/kg resorcinol in purified water from GD 6 to 19 (Unpublished study report, 2004b). Animals were fed with A04 C pelleted maintenance diet, with an iodine content of 0.3 mg/kg. Slight maternal toxicity was observed with a statistically significant decrease ($p<0.05$, -19%) in the net body weight change (reflecting maternal body weight change independently of the gravid uterus weight). No effect was observed on the number of corpora lutea, implantations, foetal viability and foetal malformations. The mean male and female foetus weights were significantly greater at 250 mg/kg/day, when compared to the controls (3.83±0.22 vs 3.67±0.18 in controls) but the values are in the historical control data range (3.6 to 3.9 g). Observations related to skeletal variations are reported in Table 14 below. There was a significant increase in the incidence of foetuses with an incompletely ossified interparietal at 40 and 80 mg/kg/day, when compared to controls ($p<0.05$ and $p<0.01$, respectively). The incidence of incompletely ossified parietals was also significantly greater at 80 mg/kg/day, when compared to controls ($p<0.05$). In the absence of any effects at 250 mg/kg/day, the relation to treatment is uncertain. There was a significantly greater incidence of incompletely ossified 5th sternebra at 250 mg/kg/day, when compared to controls ($p<0.01$). However, considering that the incidence of unossified 5th sternebra was (not significantly) lower, overall it cannot be seen as a general delay in ossification of sternebra.

Table 14: Statistically significant skeletal effects observed in Sprague-Dawley rats (unpublished study report, 2004b)

FOETAL SKELETAL VARIATIONS				
	Resorcinol dose (mg/kg bw/day)			
	0	40	80	250
Litters evaluated (number)	24	23	24	24
Foetuses evaluated (number)	170	164	164	176
Incomplete ossif. of INTERPARIETAL				
Foetal incidence (number)	6	**18***	**21****	2
(%)	3.5	11.0	12.8	1.1
Litter incidence (number)	4	7	8	2
(%)	16.7	30.4	33.3	8.3
Affected foetuses/litter (Mean% ± SD)	3.5±8.8	11.3±21.2	12.4±23.2	1.2±4.0
Incomplete ossification of PARIETAL				
Foetal incidence (number)	1	3	**8***	0
(%)	0.6	1.8	4.9	0.0
Litter incidence (number)	1	3	5	0
(%)	4.2	13.0	20.8	0.0
Affected foetuses/litter (Mean% ± SD)	0.5±2.6	1.9±5.0	**4.8•**±10.9	0.0
Incomplete ossif. of 5th STERNEBRA				
Foetal incidence (number)	118	126	128	**144***
(%)	69.4	76.8	78.0	81.8
Litter incidence (number)	24	23	24	24
(%)	100	100	100	100
Affected foetuses/litter (Mean% ± SD)	69.1±24.8	76.6±19.9	78.5±19.2	81.9±19.6
Unossified 5th STERNEBRA				
Foetal incidence (number)	42	27	24	17
(%)	24.7	16.5	14.6	9.7
Litter incidence (number)	18	16	16	11
(%)	75.0	69.6	66.7	45.8
Affected foetuses/litter (Mean% ± SD)	24.1±23.2	17.1±16.0	14.1±15.3	**9.6•**±13.8

* $p < 0.05$ and ** $p < 0.01$ (Fishers exact test); • $p < 0.05$ (ANOVA + Dunett-test)

A teratogenicity study was conducted with New Zealand White rabbits (n=20-26/group) that were exposed to 0, 25, 50 or 100 mg/kg/d resorcinol in distilled water from GD 6 to 18 (Unpublished study report, 1982b). Animals were fed with Ssniff R rabbit diet (no information on iodine content). The study was performed according to the guideline available at that time and similar to the current OECD TG 414 except that exposure was stopped early (GD 19) (ToxRtool score 1). Maternal toxicity was limited to a transient reduction of body weight gain from day 6 to 18 in high-dose females. No clinical signs and no maternal mortality were observed in relation to treatment. No effect was observed on the number of corpora lutea, implantations, foetal viability, foetal weight and foetal malformations and variations. Post-implantation loss was slightly elevated at 25 and 50 mg/kg/d (8.0%, 11.7%, 14.2% and 6.5% in groups exposed to 0, 25, 50 or 100 mg/kg/d, respectively) but the total number of intra-uterine deaths was within historical control ranges of the laboratory.

Maternal thyroid weight, histology or hormones were not examined in these prenatal development studies.

Table 15: Overview of studies via gavage

STUDIES BY GAVAGE				
Study	**ToxRtool score**	**Effects on thyroid**	**Other effects**	**Reference**
Repeated-dose toxicity and carcinogenicity studies				

STUDIES BY GAVAGE				
Study	**ToxRtool score**	**Effects on thyroid**	**Other effects**	**Reference**
$B6C3F_1$ mice (n=10/sex/group) (NIH-07 diet: 3.37 mg/kg I) 0, 28, 56, 112, 225 or 420 mg/kg/d (in deionised water) 90-day study	ToxRtool 1	No microscopic effect. Thyroid weight and thyroid hormones not investigated	Mortality and clinical signs in high-dose animals. Decreased absolute and relative adrenal weights in M at all doses.	NTP 1992
$B6C3F_1$ mice (n=60/sex/group) (NIH-07 diet: 3.37 mg/kg I) 0, 112 or 225 mg/kg/d (in deionised water) 2-year study	ToxRtool 1	No microscopic effect. Thyroid weight and thyroid hormones not investigated	Decreased body weight from week 85 in high-dose F. Clinical signs after dosing.	NTP 1992
F344/N rats (n=10/sex/group) (NIH-07 diet: 3.37 mg/kg I) 0, 32, 65, 130, 260 or 520 mg/kg/d (in deionised water) 90-day study	ToxRtool 1 Limitation: no information on standardications of blood collection for hormone analysis. This limitation do not affect the overall reliability of the study.	No microscopic effect. No effect on T3 and T4 (measured in controls and animals exposed to 130 mg/kg/d) Thyroid weight not investigated.	Mortality and clinical signs in high-dose animals. Increased absolute and relative adrenal weights in M at all doses. Increased absolute and relative liver weights in M≥130 mg/kg/d and in F≥65 mg/kg/d.	NTP 1992
F344/N rats (n=60/sex/group) (NIH-07 diet: 3.37 mg/kg I) M: 0, 112 or 225 mg/kg/d F: 0, 50, 100 or 150 mg/kg/d (in deionised water) 2-year study	ToxRtool 1	No microscopic effect. Thyroid weight and thyroid hormones not investigated	Decreased body weight and reduced survival at termination in high-dose M and F. Clinical signs after dosing in M≥112 mg/kg/d and F≥100 mg/kg/d.	NTP 1992
SD rats (n=10/sex/group) (A04 C maintenance diet: 0.3 mg/kg I) 0, 40, 80 or 250 mg/kg/d (in purified water) 90-day study	ToxRTool 1	Decreased relative thyroid weight in F at 250 mg/kg/d. No effect on thyroid histopathology. Thyroid hormones not investigated	Clinicals signs and reduced BW in F (wk 4-8) at 250 mg/kg/d. Increased landing foot splay in F ≥ 80 mg/kg/d.	Unpublished study report, 2004a
Prenatal toxicity studies				
SD rats	ToxRTool 1	Not investigated.	Maternal toxicity:	Unpublishe

STUDIES BY GAVAGE				
Study	**ToxRtool score**	**Effects on thyroid**	**Other effects**	**Reference**
(n=23 dams/group) (ND on I content in diet) 0, 40, 80 or 250 mg/kg (in distilled water) GD 6 to 15			transient reduction of BWG in high dose females from GD 6 to 15 (not statistically significant). Developmental toxicity: increased incidence of skeletal variations: 2%, 7.7%, 8.5% and 10.5% in groups exposed to 0, 40, 80 or 250 mg/kg/d, no information on statistical significance).	d study report, 1982a
SD rats (n=13 dams/group) (Purina Laboratory Rodent Chow diet: 1 mg/kg I) 0, 125, 250 or 500 mg/kg (in propylene glycol) GD 6 to 15	ToxRtool 3 Minor limitations: ▪ absence of information on purity of test substance. Major limitation: ▪ very limited presentation of experimental results.	Not investigated.	Maternal toxicity: maternal body weight gain was reduced in high dose females, although not statistically significantly. Developmental toxicity: no teratogenicity detected.	DiNardo 1985
SD rats (n=24 dams/group) (A04 C maintenance diet: 0.3 mg/kg I) 0, 40, 80 or 250 mg/kg (in purified water) GD 6 to 19	ToxRTool 1	Not investigated.	Maternal toxicity: significant decrease ($p<0.05$, -19%) in the net body weight change in high-dose females Developmental toxicity: increased foetal weight at high dose but within HCD; increased incidence of incompletely ossified parietals and interparietals but without dose-response.	Unpublished study report, 2004b
NZ rabbits (n=20-26 dams/group) (ND on I content in diet) 0, 40, 80 or 250 mg/kg (in distilled water) GD 6 to 15	ToxRTool 1	Not investigated.	Maternal toxicity: transient reduction of BW gain in high dose females from GD 6 to 19. Developmental toxicity: slightly increased incidence of post-implantation loss at 25 and 50 mg/kg/d (8.0%, 11.7%, 14.2% and 6.5% in groups exposed to 0, 25, 50 or 100 mg/kg/d, respectively). Total intra-uterine deaths within HCD.	Unpublished study report, 1982a

ND: no data; I: iodine

4.4.2.5. Diet or drinking water administration – repeated exposure

Repeated-dose toxicity studies

Three studies were conducted in rats with resorcinol administered in drinking water or diet with a specific focus on thyroid function.

In a poorly reported study, Berthezene *et al.* (1979) (ToxRtool score 3) exposed rats (strain, sex and number not specified) to 5% resorcinol in diet for two weeks. No information is available on dietary iodine intake of animals. Doses were estimated to be 2 000 mg/kg/d and 2 500 mg/kg/d for males and females, respectively, using default values recommended in table R.8-17 of ECHA R.8 guidance[12] for food consumption. **A statistically significant increase in thyroid weight was observed** (14.2±0.6 mg vs 11.5±0.3 mg, p=0.001). It is not known whether thyroid were examined histopathologically. **Radioactive labelled intrathyroid MIT/DIT ratio** (1.5±0.1 vs 0.5±0.02, p<0.001) **and T3/T4 ratio** (0.42±0.01 vs 0.21±0.02, p<0.001) **were significantly increased**. **Plasma T4 level** (2.4±0.2 vs 5.1 ±0.3 µg/100 mL, p<0.001) **and T4 half-life** (13.4±0.9 vs 18.8±1.2h, p<0.001) we**re decreased**. **No significant change was observed in the iodine level, in the percentage of free T4 and in T3 half-life**.

Resorcinol was also given in the diet during 5 days in rats with a low thyroglobulin content after a 15-day pretreatment with propylthiouracil. **Resorcinol significantly increased the TSH plasma level as well as the thyroglobulin/DNA ratio**. An increase in the level of radioactive iodine in the thyroid was also observed and may indicate a reduction of release. **This was not observed after a dietary exposure to resorcinol for one month (no other results were presented for this part of the study)**. The authors concluded that resorcinol has a weak anti-thyroid activity but may act through multiple modes of action: inhibition of iodine uptake by the thyroid, **inhibition of iodination of thyroglobulin, inhibition of conversion of iodothyrosines in iodothyronines and a transient inhibition of hormonal release**. The metabolism or transformation of circulating T4 may also be accelerated (see also section 4.4.2.1 and 4.5.1 for single iv administration and *in vitro* study).

In Cooksey *et al.* (1985) (ToxRtool score 2), female Wistar rats (n=5/group) received daily 9 µmol of resorcinol in their drinking water for 30 days (estimated dose of 9.9 mg/kg/d if entirely consumed). Animals were sacrificed 3 h after intraperitoneal injection of 1.0 µCu of ^{125}I. Relative thyroid weight of treated rats was statistically significantly higher (p<0.05) compared to controls with a doubling of values. Statistically higher I/T3+ T4 ratio (p<0.02) and lower T3+T4 concentrations (p<0.05) were observed and indicated a **decreased ability of their thyroids to incorporate ^{125}I into the active thyroid hormones** (see also section 4.5.1 for *in vitro* study). In this study, animals were fed with a low-iodine and low-protein diet (no further information on iodine level). Effects were significant compared to controls fed with a similar diet. Therefore, the diet cannot be responsible for the effects observed in resorcinol-treated animals. However, these conditions may have contributed to potentiate the effect of resorcinol on thyroid as compared to studies using higher dietary intake.

Seffner *et al.* (1995) (ToxRtool score 2) exposed cross-bred rats (n=12/group/sex) to 0.004% resorcinol in drinking water for 12 weeks. Animals were fed a diet with an iodine content of 0.9 mg/kg. Doses were estimated to be 2.56 mg/kg/d and 2.92 mg/kg/d for

[12] Guidance on information requirements and chemical safety assessment Chapter R.8: Characterisation of dose [concentration]-response for human health. ECHA. Version: 2.1 November 2012. https://echa.europa.eu/documents/10162/13632/information_requirements_r8_en.pdf/e153243a-03f0-44c5-8808-88af66223258

males and females, respectively, using default values recommended in table R.8-17 of ECHA R.8 guidance[13] for water consumption. All organs were weighed and thyroid glands were histologically examined. No information on thyroid weight was reported. The following statistically significant changes were observed in male and in female thyroid tissues: **increase in the height of epithelial (follicular) cells (+12.6%), decrease of mean diameter of follicles (-16%), decrease in the follicle epithelium index (follicle diameter/epithelial height), which is indicative of an increased follicular cell activity and loss of colloid.**

Reproductive toxicity study

A two-generation study was performed in Crl: CD(SD) rats via drinking water.

In the preliminary study (Unpublished study report, 2003, ToxRtool score 1), resorcinol was administered in drinking water at concentrations of 0, 10, 40, 120 and 360 mg/l to Sprague Dawley rats (14/sex/group) for 28 days prior to mating, during mating, throughout gestation and lactation until euthanasia (doses in Table 16). Animals were fed with Rodent LabDiet® 5002, with an iodine content of 0.98 mg/kg. Administration of resorcinol to F1 pups selected for exposure (one pup/sex/litter) began at weaning on PND 21 and F1 pups received resorcinol through euthanasia on PND 28. The F1 pups selected for behavioural testing did not receive direct exposure to resorcinol following weaning on PND 21. Behavioural evaluations (three F1 pups/sex/litter) included FOB at PND 21, and acoustic startle response at PND 20 and 60, which measures reflex startle reaction in response to an auditory stimulus, known to be mediated primarily by neurons of the reticular formation in the brainstem. The two other behaviours measured were spatial memory (Biel maze swimming test at PND 22 and 62) and locomotor activity (at PND 21 and 61), known to be regulated by thyroid hormones and involving the hippocampus/cortex or several brain and spinal regions, respectively (Zoeller and Rovet, 2004).

Table 16: Doses (mg/kg/d) in the preliminary reproductive toxicity study (Unpublished study report, 2003)

Dose (mg/L)	0	10	40	120	360
F0 MALES					
Prior to breeding	0	0.8	3.9	13.1	36.9
After breeding	0	0.6	2.8	10.5	31.4
F0 FEMALES					
Prior to breeding	0	0.8	5.1	15.6	46.6
During gestation	0	1.3	5.1	14.6	44.0
During lactation	0	5.0	18.5	58.7	174.4
F1 PUPS					
PND21-28	0	5.0	18.5	58.7	174.4

Hormone analyses for TSH, total T4 and total T3 were conducted at the interim necropsy on F0 males (7/group), at the scheduled necropsy on all F0 parental animals (7 males/group, 14 females/group), on PND 28 for F1 pups selected, and on PND 4 for all culled F1 pups (pooled without regard to sex). Thyroid glands from all surviving F0 parental animals were examined microscopically. Brain measurements of size were conducted on all F1 pups exposed to resorcinol or selected for behavioural testing. A qualitative histopathological analysis of the brain (forebrain, midbrain, hindbrain) after staining of slides with hematoxylin and eosin was conducted for the aforementioned animals in the control and 360 mg/L groups.

[13] Guidance on information requirements and chemical safety assessment Chapter R.8: Characterisation of dose [concentration]-response for human health. ECHA. Version: 2.1 November 2012. https://echa.europa.eu/documents/10162/13632/information_requirements_r8_en.pdf/e153243a-03f0-44c5-8808-88af66223258

In F0 males and females, no effect was observed on survival and body weight. No clinical signs were noted.

In F0 males, an elevated TSH level (>30%) was observed in males at interim sacrifice (following breeding) and was +42% in the group exposed to 360 mg/l. Statistical significance was not reached (p=0.195). **Minimal follicular cell hyperplasia was observed in 5/7 and 6/7 animals of the groups exposed to 120 and 360 mg/l**, respectively (3/7 in controls). A minimal decrease of colloid was observed in 3/7 animals exposed to 360 mg/l vs 1/7 in controls. However, none of these parameters were statistically different from controls. **No statistically significant effect on thyroid weight was observed**.
In F0 males sacrificed after weaning of F1 pups, an elevated absolute thyroid weight was observed from 120 mg/l. Minimal follicular cell hyperplasia was observed in 5/7 animals exposed to 360 mg/l vs 3/7 in controls). However, none of these parameters were statistically different from controls. It is noted that this preliminary study was performed on a low number of animals that limits its statistical power.

In F0 females**, an increase in T3 was observed with statistical significance reached at the high dose**. **No effect was observed on T4, TSH or on thyroid weight**. **6/14 females exposed to the high dose have minimal follicular hyperplasia vs 3/13 in controls** and one additional female had mild follicular hyperplasia vs none in controls.

Table 17: Observations related to thyroid in F0 in the preliminary reproductive toxicity study (Unpublished study report, 2003)

Dose (mg/L)	0	10	40	120	360
F0 MALES					
Interim necropsy (following breeding) (n=7/group)					
TSH (ng/mL)	11.96±3.091	13.66±4.087	16.59±4.727	15.56±5.454	17.03±6.724
T3 (ng/dL)	90.10±23.974	88.13±16.405	87.77±16.574	86.64±16.302	96.89±24.430
T4 (µg/dL)	5.79±1.439	6.71±1.154	5.63±0.929	6.21±1.163	6.13±1.840
Abs. thyroid wt (mg)	24.3±10.94	32.2±4.99	23.0±2.79	30.2±3.19	27.0±4.06
Follicular cell hypertrophy (min.)	0/7	0/7	0/7	0/7	1/7
Follicular cell hyperplasia (min.)	3/7	1/7	3/7	5/7	6/7
Decreased colloid (min.)	1/7	0/7	0/7	0/7	3/7
Final necropsy (after weaning of F1 pups) (n=6-7/group)					
TSH (ng/mL)	17.53±5.373	14.27±4.793	20.17±5.172	15.33±3.943	20.03±6.536
T3 (ng/dL)	136.90±23.496	142.86±29.600	117.23±14.17	130.86±17.535	131.14±19.701
T4 (µg/dL)	6.23±0.687	5.94±1.620	5.93±0.742	5.87±1.029	5.40±1.120
Abs. thyroid wt (mg)	25.4±3.28	24.9±3.88	24.6±3.01	28.1±3.42	28.6±4.20
Follicular cell hypertrophy (min.)	0/7	0/7	0/7	0/7	2/7
Follicular cell hyperplasia (min.)	3/7	1/7	4/7	3/7	5/7
Decreased colloid (min.)	0/7	1/7	1/7	0/7	0/7
F0 FEMALES					
Scheduled necropsy (after weaning of F1 pups) (n=12-14/group)					
TSH (ng/mL)	14.14±6.639	15.27±7.381	13.20±3.660	14.62±4.020	15.18±4.000
T3 (ng/dL)	69.27±17.838	73.60±16.474	72.28±16.113	80.76±13.513	**87.85*±17.336**
T4 (µg/dL)	3.90±1.149	3.73±0.848	4.04±1.185	4.05±0.972	4.13±0.890
Abs. thyroid wt (mg)	23.2±5.58	22.2±3.82	23.6±4.30	24.3±2.81	22.8±5.21
Follicular cell hyperplasia (min.)	3/13	2/14	2/12	1/13	6/14[a]
Decreased colloid (min.)	1/13	1/14	2/12	3/13	2/14

Mean±SD; [a] one additional animal with mild hyperplasia; min: minimal grade

Length of estrous cycle, fertility index, gestation length, number of implantation sites,

litter size, pups survival, offspring body weight were unaffected by treatment.

In F1 pups sacrificed at PND 4, T3 levels were elevated and the increase was > 20% compared to controls in the group exposed to 360 mg/l but the difference was not statistically significant (p=0.291). **An elevated level in T4** was also noted in groups exposed to 120 mg/l (+34%, p=0.053) and 360 mg/l (+27%, p=0.156).

F1 pups that were further exposed to resorcinol until PND 28 exhibited no clinical signs. Body weights and food and water consumption were unaffected. **Serum thyroid hormones and thyroid weights were not significantly modified in the exposed groups at PND 28. A dose-related increase in T3 was observed in females that reached +12% in the group exposed to 360 mg/l (p=0.177)**. No significant effect was observed on brain weight, length or width. No changes in hematoxylin/eosin staining were identified upon examination of the forebrain, midbrain and hindbrain in the high-dose group.

Table 18: Observations related to thyroid in F1 in the preliminary reproductive toxicity study (Unpublished study report, 2003)

Dose (mg/L)	0	10	40	120	360
F1 PUPS (PND4) (n=12-14/group)					
TSH (ng/mL)	7.87±1.359	8.00±2.193	7.67±1.442	9.53±4.711	8.04±1.412
T3 (ng/dL)	30.82±9.573	28.49±7.101	31.21±9.920	33.27±9.013	37.19±12.588
T4 (µg/dL)	1.10±0.288	1.23±0.362	1.30±0.269	1.47±0.425	1.40±0.512
F1 MALES (PND28) (n=12-14/group)					
TSH (ng/mL)	5.82±0.797	7.07±4.246	7.24±1.482	6.70±1.375	7.32±2.457
T3 (ng/dL)	135.62±20.642	132.38±10.681	139.77±29.679	141.18±26.563	137.21±21.265
T4 (µg/dL)	3.55±0.692	3.68±0.634	3.38±0.554	3.52±0.760	3.32±0.574
Abs. thyroid wt (mg)	12.2±2.61	12.8±2.12	12.1±2.57	13.1±2.82	11.8±2.30
F1 FEMALES (PND28) (n=12-14/group)					
TSH (ng/mL)	6.37±1.153	5.99±1.710	6.65±1.285	6.84±1.697	6.91±1.403
T3 (ng/dL)	121.86±14.927	128.93±14.615	130.43±25.608	133.43±24.496	136.71±18.685
T4 (µg/dL)	3.48±1.025	3.39±0.613	3.44±0.588	3.69±0.930	3.26±0.649
Abs. thyroid wt (mg)	12.0±3.16	12.9±2.83	12.0±2.65	11.6±3.03	12.1±2.35

Mean±SD

In F1 pups selected for behavioural analyses, body weights were not affected. The mean day of acquisition of balanopreputial separation was significantly increased in the 360 mg/L group (44.0 days vs 42.2 days) but this is comparable to the mean value in the laboratory historical control dataset (mean: 44.5 days; range: 41.6-49.0 days). No effect was observed in females on the day of vaginal opening. No effect was observed on males and females around PND-21 or PND60 on acoustic startle response and spatial memory (Biel maze swimming test). For locomotor activity in males, no effect was described in young animals (PND21). **A significantly increased cumulative activity was observed around PND60 during the 60 min of the test at 40, 120 and 360 mg/L (+34%, +38%, +41% above the control group, respectively, calculated on the basis of data given in the report)**.

The figure below illustrates the obtained data for locomotor activity in PND60 males. The authors statistically analysed the cumulative activity, but not the time-dependent decrease of activity per 15 min. In both cases, a resorcinol dose-dependent increase of activity was observed.

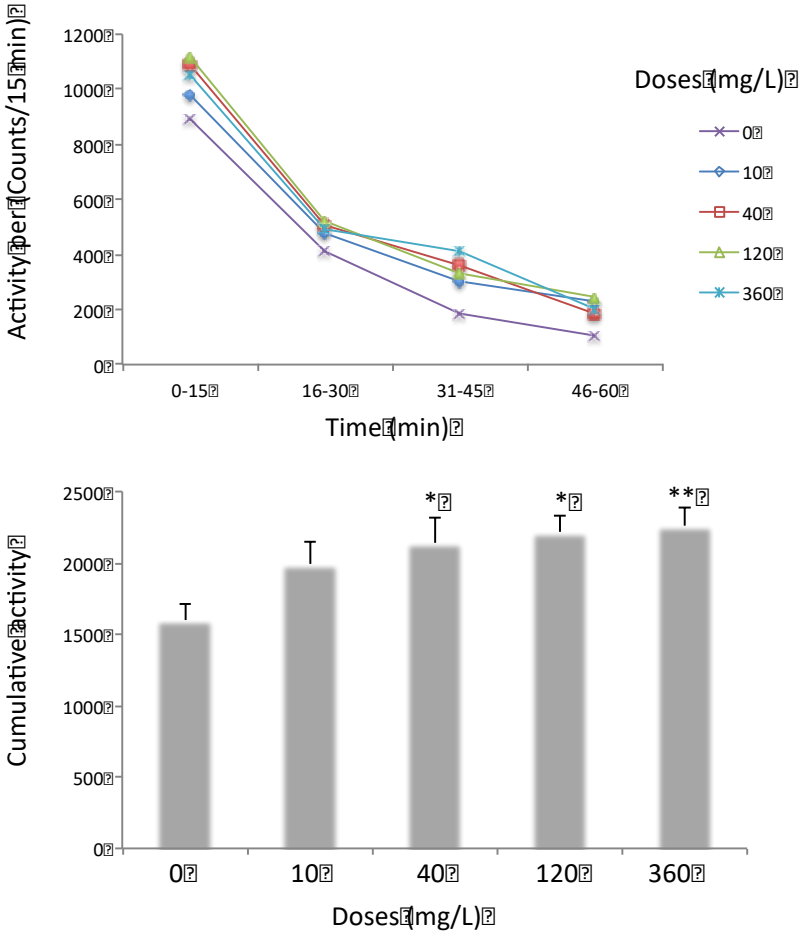


Figure 1: preliminary reproduction study - locomotor activity in PND 60 males (F1)

In females, a tendency of increase in locomotor activity was observed at PND60, but it is not statistically significant (see Figure 2 below). The behavioural analyses in females did not take into account the stage of the estral cycle which can be a source of variability within a group.

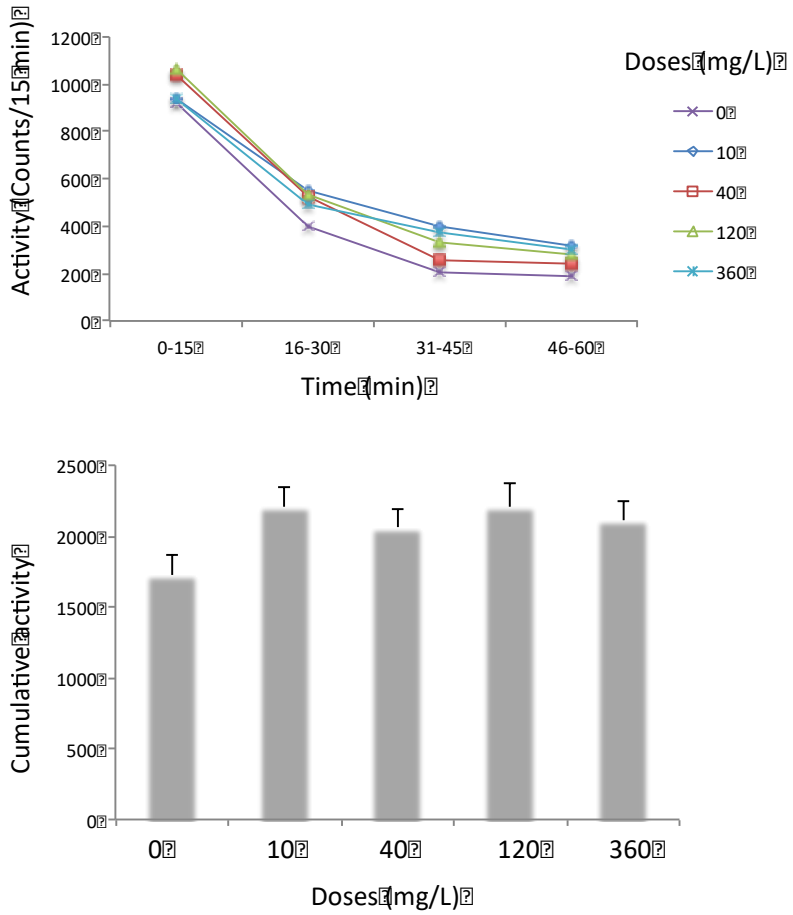


Figure 2: preliminary reproduction study - locomotor activity in PND 60 females (F1)

The main study (Unpublished study report, 2005a, Welsch *et al*., 2008a, ToxRtool score 1) was performed according to OECD TG 416 for two-generation study. Resorcinol (purity 100 %) was administered in drinking water at concentrations of 0, 120, 360, 1000 and 3000 mg/L (doses in mg/kg/d in Table 19) to Sprague Dawley rats (30/sex/group) for at least 70 days prior to mating, then during the mating period and finally during gestation, lactation and weaning. Animals were fed with Rodent LabDiet® 5002, with an iodine content of 0.98 mg/kg.

Table 19: Doses in mg/kg/d in the two-generation reproductive toxicity study (Unpublished study report, 2005a)

ConcentrationConcentration (mg/L)	0	120	360	1000	3000
F0 ANIMALS					
Males prior to mating	0	11	33	88	246
Males after mating	0	8	23	62	177
Females prior to mating	0	17	51	123	294
Females during gestation	0	14	48	110	278
Females during lactation	0	31	98	245	674
F1 ANIMALS					
Males prior to mating	0	14	41	115	304
Males after mating	0	8	23	67	173
Females prior to mating	0	18	48	141	347
Females during gestation	0	14	43	117	296
Females during lactation	0	28	85	237	645

Thyroid hormone analysis (i.e. T3, T4 and TSH) and bioanalysis to determine plasma

resorcinol concentration were conducted as part of a study design. Blood samples were collected and analysed from 15 randomly selected F1 parental animals/sex/group during the week prior to necropsy.

For analysis of total T3, T4 and TSH, the blood samples ewre collected via the vena cava from 15 randomly selected F0 and F1 parental animals/sex/group at the scheduled necropsies following weaning of the pups. In F1 and F2 pups, blood was collected from all culled pups from 15 randomly selected litters on PND 4 (samples were pooled without regard to sex) and from one pup/sex from 15 randomly selected litters on PND 21. The time of sampling in the day was variable. Blood sampling was generally performed between 8:45 AM and 1:45 PM in PND4 pups, between 9:15 AM and 4:00 PM in PND21 animals and between 9:30 and 5:30 PM in adults.

The organs including thyroid gland from all F0 and F1 parental animals were weighed at the scheduled necropsies. For microscopic examination, selected tissues including the thyroid gland from all F0 and F1 parental animals in the control and high concentration level (3000 mg/l) groups and all animals found dead or euthanised in extremis, were examined. The thyroids of all F0 animals in the 1000 mg/l group were also examined. In addition, a stereomicroscopy was done on 15 randomly selected F0 parent animals per sex in the control and 3000 mg/l groups and 15 randomly selected F0 parent males in the 1000 mg/l. Brains were not examined histologically.

In F0 males and females, no significant effect is observed on survival. No clinical signs were noted. Reduced body weights were observed in males and females at the highest dose and were related to a decreased water consumption. The reduction is statistically significant in females during lactation up to final sacrifice after F1 weaning.

In F0 males, no significant change in thyroid hormones was observed. Elevated TSH (+35%, p=0.29) and T3 (+12%, p=0.25) levels were noted at high dose but were not statistically significant. Absolute thyroid weight was elevated but not significantly and it was due to a single male with enlarged thyroid and bilateral benign follicular cell adenomas. After exclusion of this animal, the mean thyroid weight was 26.7 mg and was similar to controls. The study report concluded that a single occurrence of bilateral benign follicular cell adenoma can be of spontaneous origin. This is supported by the absence of hyperplasia observed in exposed animals but no historical control data from the concurrent laboratory were provided. **A statistically significant decrease in colloid in the 3000 mg/L group F0 males was observed by objective morphometric evaluation conducted in 15 animals randomly selected in the control and 3000 mg/l groups**. The mean percent colloid was decreased in the 3000 mg/L group F0 males by 11.09% compared to the control group, and this decrease is statistically significant. Other findings in F0 males were an increase in mean liver and kidney weights relative to final body weight in the 3000 mg/L group males ($p<0.05$) compared to the control group.

In F0 females, **no significant changes in thyroid hormones, no effect on thyroid weight and no histopathological findings were observed**. In contrast to F0 males, mean colloid in the 3000 mg/L group F0 females was reduced by 3.55% compared to the controls, and in a non statistically significant manner. The mean estrous cycle length was slightly but significantly reduced in the 1000 mg/L group (4.1 days vs 4.4 in the controls) but 4 days was considered to be a normal length in rats by the study submitter (no data on historical control data given).

Fertility index, gestation length, sperm motility, concentration and morphology, number of implantation sites and litter size were unaffected by treatment.

Table 20: Observations related to thyroid in F0 in the two-generation main study (mean±SD) (Unpublished study report, 2005a)

Dose (mg/L)	0	120	360	1000	3000
F0 MALES - Scheduled necropsy (after weaning of F1 pups) (n=15/group for hormones; n=30/group for histo.)					
TSH (ng/mL)	9.3±3.88	9.6±3.38	11.3±4.46	11.8±5.55	12.6±8.7
T3 (ng/dL)	131.38±21.124	133.31±28.835	124.79±18.066	143.33±25.351	147.17±28.849
T4 (µg/dL)	6.11±1.279	6.19±1.297	5.87±1.247	5.91±.952	5.45±1.108
Abs. thyroid wt (mg)	26.9±4.65	25.2±5.58	25.0±4.50	25.9±5.16	37.1 [a] ±57.11
Decreased colloid (min.)	2/30	NA	NA	2/30	7/30
Follicular cell adenoma)	0/30	NA	NA	0/30	1/30
F0 FEMALES - Scheduled necropsy (after weaning of F1 pups) (n=15/group for hormones; n=30/group for histo.)					
TSH (ng/mL)	9.4±3.47	8.3±2.26	9.4±3.51	7.9±2.69	9.8±3.30
T3 (ng/dL)	136.73±27.385	141.11±34.606	133.00±28.442	146.33±24.348	138.27±19.455
T4 (µg/dL)	4.82±0.941	4.84±1.387	4.80±1.248	4.74±1.418	4.21±0.807
Abs. thyroid wt (mg)	22.0±5.85	21.4±3.68	20.6±3.94	22.2±3.31	21.9±4.62
Decreased colloid (min.)	3/30	NA	NA	6/29	4/30

Mean±SD; NA: not applicable; min: minimal grade; [a]: 26.7mg after exclusion of the animal with bilateral follicular cell adenoma

In F1, sex ratio, pup survival and offspring body weight were unaffected by treatment.

In F1 pups sacrificed at PND 4, no significant changes in thyroid hormones were observed.

In F1 pups sacrificed at PND 21, **a significant increase in TSH was observed in male groups exposed to 360 and 3000 mg/l** (no dose-response). **Thyroid weight was unaffected by treatment**. Other findings were restricted to statistically significantly increased relative (to final body and brain weight) thymus weights in the 3000 mg/L group females. No effect was observed on acquisition of balanopreputial separation or vaginal opening.

Mean body weights were not significantly reduced in high-dosed F1 males (5.7% to 7.1%) from the mating period until euthanasia and in high dose F1 females from lactation and after (up to 6.1%). The effect corresponded to decreased water consumption. F1 fertility index, gestation length, sperm motility, concentration and morphology, number of implantation sites and F2 litter size were unaffected by treatment.

In F1 animals sacrificed after weaning of F2**, no significant effect was identified in thyroid hormone levels. No effects on thyroid weight and histology were observed**. Mean liver weight relative to final body weight in the 3000 mg/L group males was increased (statistically significant, $p<0.05$) compared to the control group. Blood samples were taken in F1 animals after 143 to 155 days of dosing (1 week prior necropsy) and blood resorcinol levels were detected only in few animals (1/10 M and 2/10 F) in the 3000 mg/L group (116 to 621 ng/mL resorcinol) (LOQ=0.1 µg/mL) (see section 4.3).

Table 21: Observations related to thyroid in F1 in the two-generation main study (mean±SD) (Unpublished study report, 2005a)

Dose (mg/L)	0	120	360	1000	3000
F1 PUPS (PND4) (n=15/group)					
TSH (ng/mL)	5.5±3.71	4.7±1.07	4.5±0.73	4.9±0.68	4.7±0.73
T3 (ng/dL)	37.21±7.390	37.67±6.376	35.15±6.403	34.19±6.733	39.25±6.382
T4 (µg/dL)	0.70±0.423	0.79±0.241	0.62±0.224	0.85±0.288	0.81±0.212
F1 MALES (PND21) (n=14-15/group for hormones; n=25-30/group for histo.)					
TSH (ng/mL)	3.6±1.66	3.8±1.64	**5.4*±2.09**	3.9±1.54	**5.6*±2.31**
T3 (ng/dL)	123.57±19.421	147.10±37.197	128.40±18.236	155.13±24.089	138.84±29.090
T4 (µg/dL)	4.29±1.111	4.47±1.359	3.93±0.956	4.39±1.442	3.92±0.882
Abs. thyroid wt (mg)	5.1±1.30	5.5±1.11	5.7±0.84	5.7±1.12	5.5±1.15
F1 FEMALES (PND21) (n=14-15/group for hormones; n=25-30/group for histo.)					

TSH (ng/mL)	2.3±1.50	3.1±1.60	3.7±3.13	2.3±1.52	3.4±1.83
T3 (ng/dL)	129.16±30.595	137.67±21.550	127.31±25.949	135.43±29.406	122.98±18.995
T4 (µg/dL)	4.01±0.974	4.28±1.030	4.17±0.774	4.03±1.077	3.60±0.656
Abs. thyroid wt (mg)	6.0±1.01	5.8±1.20	5.9±0.93	5.3±1.21	5.5±1.30
F1 MALES (scheduled sacrifice) (n=15/group for hormones; n=29-30/group for histo.)					
TSH (ng/mL)	12.1±5.10	13.5±5.92	12.1±4.56	13.2±5.79	14.3±5.25
T3 (ng/dL)	158.33±26.038	152.80±26.793	150.93±24.543	162.73±25.277	170.93±31.167
T4 (µg/dL)	5.42±0.820	5.96±1.209	5.12±0.865	4.99±0.836	5.31±0.752
Abs. thyroid wt (mg)	29.6±6.88	27.6±9.09	28.9±7.44	26.9±6.40	29.2±7.37
Decreased colloid (min.)	6/29	NA	NA	NA	4/30
F1 FEMALES (schedules sacrifice) (n=15/group for hormones; n=30/group for histo.)					
TSH (ng/mL)	11.1±5.29	9.0±3.57	9.7±2.57	9.9±1.64	9.6±2.85
T3 (ng/dL)	140.33±36.731	149.00±30.589	146.87±19.261	149.00±18.237	133.74±30.412
T4 (µg/dL)	3.83±1.069	4.10±1.298	3.87±1.190	4.10±0.883	3.09±0.966
Abs. thyroid wt (mg)	24.6±6.15	23.6±5.90	23.5±5.25	24.6±5.75	24.5±5.40
Decreased colloid (mild)	3/30	NA	NA	NA	4/30

Mean±SD; NA: not applicable; min: minimal grade; * p<0.05

In F2, sex ratio, pups survival and offspring body weight were unaffected by treatment.

In F2 pups sacrificed at PND 4, no significant changes in thyroid hormones were observed.

In F2 pups sacrificed at PND 21, changes weare observed in the group exposed to 1000 mg/l but not at the highest dose. **They consist of a statistically significant decrease in T4 (-23%) as well as a statistically significant increase in absolute thyroid weight in females**. The increase in thyroid weight is not significant when relative weight to final body weight is considered but is statistically significant at 360 and 1000 mg/l and dose-related when relative weight to brain weight is considered. No significant changes were observed in males. However, **T4 levels were similarly low (-19%, p=0.184) and thyroid weight elevated at the dose level of 1000 mg/l**. No noticeable macroscopic findings were reported at necropsy. No microscopic examination was performed.

Table 22: Observations related to thyroid in F2 in the two-generation main study (mean±SD) (Unpublished study report, 2005a)

Dose (mg/L)	0	120	360	1000	3000
F2 PUPS (PND4) (n=15/group)					
TSH (ng/mL)	4.8±1.34	4.6±1.61	5.1±1.41	4.9±0.99	4.9±1.49
T3 (ng/dL)	53.49±13.999	47.22±8.961	54.53±11.690	55.51±10.094	47.39±12.571
T4 (µg/dL)	1.06±0.274	0.93±0.172	1.12±0.346	1.03±0.231	1.17±0.180
F2 MALES (PND21) (n=15/group for hormones; n=23-27/group for histo.)					
TSH (ng/mL)	5.0±2.61	5.0±1.79	5.3±2.19	5.6±1.30	5.1±1.43
T3 (ng/dL)	157.59±32.663	140.75±34.024	148.22±42.440	156.43±52.652	140.91±44.695
T4 (µg/dL)	4.10±1.587	3.68±1.217	3.66±0.939	3.33±0.764	3.67±0.675
Abs. thyroid wt (mg)	5.8±1.39	6.3±1.86	6.3±1.47	7.0±2.01	6.4±1.31
F2 FEMALES (PND21) (n=14-15/group for hormones; n=22-28/group for histo.)					
TSH (ng/mL)	5.2±2.36	5.0±0.97	5.8±1.84	6.0±1.36	5.2±1.12
T3 (ng/dL)	137.325±42.11	128.44±35.299	140.85±27.386	150.99±47.072	123.06±33.836
T4 (µg/dL)	4.13±1.491	3.57±1.128	4.06±1.087	**3.22*±1.039**	3.53±0.746
Abs. thyroid wt (mg)	5.3±1.93	6.4±1.76	6.5±2.11	**7.2*±2.05**	6.4±1.70

Mean±SD; * p<0.05

Table 23: Overview of studies via diet or drinking water

STUDIES via diet or drinking water				
Study	**ToxRtool score**	**Effects on thyroid**	**Other effects**	**Reference**
Repeated-dose toxicity studies				
Rats (ND on I content in diet) 5% in diet (approx. 2 000 and 2 500 mg/kg/d for M and F, respectively) Exposure for two weeks	ToxRtool score 3 Major limitations: ▪ experimental conditions poorly described (absence of information on purity of test substance, number of animals, housing and feeding conditions, details of experimental protocol etc), ▪ limited reporting of results. Used in a WoE approach as supporting study	Increased thyroid weight. Increase in radioactive labelled MIT/DIT ratio and T3/T4 ratio. Decrease in plasma T4 level and T4 half-life. No significant change in the iodine level, in the percentage of free T4 and in T3 half life.	No information, study targeted on thyroid effects.	Berthezene, 1979
Wistar rats (5/group) fed low iodine and low-protein diet 9 µmol in drinking water (approx. 9,9 mg/kg/d) Exposure for 30 days	ToxRtool 2 Minor limitations: ▪ experimental conditions poorly described (absence of information on purity of test substance, housing conditions), ▪ low numbers of animals, ▪ limited reporting of results.	Increased relative thyroid weight (doubling compared to controls). Increased I/T3+ T4 ratio. Decreased T3+T4.	No information, study targeted on thyroid effects.	Cooksey, 1985
Rats (12/sex/group) (0.9 mg/kg I in diet) 0.004% in drinking water (approx. 2.56 and 2.92 mg/kg/d for males and females, respectively) Exposure for 12 weeks	ToxRtool 2 Minor limitations: ▪ absence of information on purity of test substance, no information on water consumption.	No information on thyroid weight. Histopathology: increase in the height of epithelial cells, decrease of mean diameter of follicles, decrease in the follicle epithelium index	No information, study targeted on thyroid effects.	Seffner, 1995
Reproductive toxicity studies				
Crl: CD(SD) rats (14/sex/group) (Rodent LabDiet® 5002: 0.98 mg/kg I) Dose range-finding reproductive toxicity study 0, 10, 40, 120, 360 mg/l in drinking water	ToxRTool 1 Limitation: blood collection for hormone analysis not standardised. This limitation can interfere with detection of hormonal changes but do not affect the overall reliability of the study.	F0: Increase in T3 in F (significant) Elevated incidence of follicular cell hyperplasia in high-dose M and F (not significant) F1 pups: Increase in T3 and T4 in pups at PND4 and increase in T3 in F at and in TSH in M at PND28 (not	F0: No effect. F1 pups: Significant increase in locomotor activity in males at PND	Unpublished study report, 2003

STUDIES via diet or drinking water				
Study	**ToxRtool score**	**Effects on thyroid**	**Other effects**	**Reference**
Corresponding in F0 animals prior to breeding to doses of 0, 0.8, 3.9, 13.1 or 36.9 mg/kg/d in M and to 0, 0.8, 5.1, 15.6 or 46.6 mg/kg/d in F, respectively (complete dose correspondences in text above)		significant)	60 (no effect on PND21) No effect on brain wt and size. No effect on acoustic startle response and spatial memory.	
Crl: CD(SD) rats (n=30 /sex/group) (Rodent LabDiet® 5002: 0.98 mg/kg I) Two-generation reproductive toxicity study (OECD TG 416) 0, 120, 360, 1000 or 3000 mg/l in drinking water Corresponding in F1 animals prior to mating to doses of 0, 14, 41, 115 or 304 mg/kg/d in M and to 0, 18, 48, 141 or 347 mg/kg/d in F, respectively (complete dose correspondences in text above)	ToxRTool 1 Limitation: blood collection for hormone analysis not standardised. This limitation can interfere with detection of hormonal changes but do not affect the overall reliability of the study.	F0: effects in males at 3000 mg/l: Elevated TSH and T3 (not significant). Decreased colloid. F1: stat. increase in TSH in PND 21 males at 360 and 3000 mg/l. F2: stat. decrease in T4 and stat increase in absolute thyroid weight in females at 1000 mg/l (similar effect in males but not significant).	F0: decreased water consumption from 1000 mg/l; decreased body weight and in M increased liver and kidney weight at 3000 mg/l F1: decreased water consumption and decreased body weight at 3000 mg/l; increased relative thymus weight in females at 3000 mg/l F2:no effects	Unpublished study report, 2005a Welsch, 2008a

ND: no data; I: iodine

4.4.2.6. **Inhalation –repeated exposure**

A subchronic study by inhalation was performed for resorcinol with albino rats (unpublished study report, 1977) (ToxRtool score 3). Resorcinol was dissolved in distilled water and administered as an aerosol. Animals were fed with Purina Laboratory Chow. An iodine content of 1 mg/kg is reported for this diet from public source.

In a 14-day preliminary study, 5 rats/sex were exposed to 220 ppm resorcinol (approximately 1000 mg/m^3). At the end of exposure all animals were examined grossly. Except slight haemorrhagic spots on the lung of one male rats, all tissues appeared essentially normal.

In the main study, 25 rats/sex were exposed to a mean concentration of 213 ppm of resorcinol for 8 h/day. 5 rats/sex served as laboratory control and 5 rats/sex as pair-fed

controls. Exposure was suspended after 64 days of exposure due to excessive mortality, 20% (n=5) in the male group and 28% (n=7) in the female group. One half of the survivors were sacrificed one week later. After a two-week pause, animals appeared to recover and exposure was initiated again for the rest of the animals until a total of 90 daily exposures (26 additional daily exposures). Since no significant differences in lesions of animals sacrificed after 64 and 90 resorcinol exposures were noted, these groups (as well as males and females) were pooled for reporting of the lesions. The high mortality was possibly caused by an infection. However, the role of resorcinol cannot be excluded as mortality affected only test groups, suspension of exposure stopped mortality in excess and histopathological examination demonstrated signs of infection in the heart, lungs and liver in only 5/12 animals that died prematurely.

Average food consumption was decreased in exposed rats. Body weight was also decreased compared to laboratory controls (statistically significant in males and females) and to pair-fed controls (statistically significant in females).

Statistically significant differences in organ weights compared to laboratory controls were observed in the liver (-32% in males), kidneys (-17% in males and 611% in females), spleen (-37% in males and -15% in females) and **adrenals (+104% in females)**. In these organs, noticeable histopathological findings were observed in the liver (portal lymphocytic infiltration in 50% of test animals vs 35% of controls) and in the spleen (focal lymphocytic infiltration in 8% and hydronephrosis in 5% of the test animals versus 0% in controls). In particular, there were no pulmonary changes attributable to treatment. Although several alveolar giant cells and macrophage clusters were observed in a few resorcinol exposed animals, similar findings were also present in control animals.

No differences in thyroid weights weres observed. However, **39% of resorcinol-treated animals (15/38) had thyroid hyperplasia** (none in controls). The hyperplasia was characterised by a great increase in the number of acini. These were small, lined by columnar epithelium enclosing a very small lumen**. The acini contained little or no colloid**. None of the control rats had this lesion. Pathologic findings in the thyroid of the test animals that died prematurely were not determined due to severe post-mortem changes.

Some differences were also observed in blood chemistry and haematological parameters (↘ glucose, ↗ RBC, ↗ Hb, ↗ haematocrit, ↘ lymphocytes) but no valid conclusions can be drawn based on them.

Although this study presents serious flaws for a regulatory study (single dose tested, poor reporting), it is considered that the study indicates effects on thyroid that can be attributed to exposure to resorcinol by inhalation.

Table 24: Overview of repeated dose toxicity study via inhalation route

REPEATED DOSE TOXICITY INHALATION ROUTE				
Study	**ToxRtool score**	**Effects on thyroid**	**Other results**	**Reference**
HLA-SD Rat male/female (25 animals/sex exposed to resorcinol, 10 controls/sex) (Purina Laboratory Chow diet: 1 mg/kg I) Subchronic	ToxRtool 3 Minor limitations: ▪ absence of information on purity of test substance, Major limitations: ▪ No information on verification of achieved concentrations ▪ Important mortality that may	There were no differences in thyroid weights. 39% (15/38 animals) of resorcinol treated animals (males and females pooled) had thyroid hyperplasia. No thyroid hyperplasia was observed in	The exposure was terminated at 64 days due to excess mortality, possibly caused by an infection. After two weeks of rest, the exposure was continued until the total of 90 exposures were completed.	Unpublished study report (1977)

REPEATED DOSE TOXICITY INHALATION ROUTE				
Study	**ToxRtool score**	**Effects on thyroid**	**Other results**	**Reference**
(inhalation) 220 ppm (1000 mg/m^3) Exposure 90 days (8 hours/day)	have interfere with detection of effects Used in a WoE approach as supporting study	controls.	Changes in organ weights (liver, kidney, adrenals, spleen). There were no pulmonary changes attributable to treatment.	

I: iodine

4.4.2.7. Summary of *in vivo* effects related to thyroid

Effects on thyroid

All experimental *in vivo* studies that provided information on thyroid weight, thyroid histology and/or thyroid hormones are discussed below and summarised in Table 25.

By subcutaneous route, effects on thyroid were observed when resorcinol was administered in aqueous solution (Cheymol *et al.*, 1951) or in oil when dose and duration were sufficient, i.e. exceeding 308 mg/kg/d for 47 days (Doniach & Logothetopoulos, 1953; Samuel, 1955). Effects were characterised by an increased thyroid weight and histological lesions consisting of depletion of colloid and in some case hyperaemia, hyperplasia of epithelial cells and variability in their sizes and shapes. The studies were generally conducted with variable conditions of exposure (doses, vehicle, duration) and low numbers of animals for each experimental conditions. Poor reporting of experimental conditions and results also affect the reliability of some of the studies. However, it is relevant to consider these studies in the weight of evidence. **The consistency in findings and concomitant observation of histopathologic lesions and thyroid enlargement indicate that the effects were not incidental and can be attributed to resorcinol**.

Few studies were conducted via dermal application. Effects similar to findings observed by the subcutaneous route were reported when applied in ointment at high concentration (12.5%) for 4 weeks by Samuel (1955). After 3 weeks of application, no effect was observed on the thyroid weight by Doniach & Logothetopoulos (1953).

By gavage, no effect on thyroid was observed in F344 rats and B6C3F1 mice after subchronic exposure up to 520 mg/kg/d and chronic exposure up to 225 mg/kg/d (NTP 1992). In Sprague-Dawley rats, a decrease in thyroid weight was however observed in females exposed at 250 mg/kg/d for 90 days, although no histological findings were present (Unpublished study report, 2004a). In contrast, increased thyroid weight was noted when exposure stopped for 4 weeks.

Water was used as vehicle in all these studies.

When administered in diet or drinking water, **an effect on thyroid weight as well as on thyroid hormones was observed** in rats after exposure to approximately 2 000/2 500 mg/kg/d for 14 days (Berthezene *et al.,* 1979) or 9.9 mg/kg/d for 30 days in rats fed with a low-iodine diet (Cooksey *et al*., 1985). Microscopic findings were investigated in Seffner *et al.* (1995) **and some changes in thyroid structure that are indicative of compensatory mechanisms** were **observed at doses as low as approximately 2.5 mg/kg/d after administration for 12 weeks.** The decrease of iodine uptake observed

in Doniach & Fraser (1950) after a short exposure to 2% resorcinol in drinking water also provides supporting evidence of thyroid effects of resorcinol via drinking water.

In a preliminary reproductive toxicity study of 2003 and a main study finalised in 2005 (unpublished study reports), **sporadic statistically significant changes** were noted:

- In the preliminary study, **an increase in T3 in F0 females exposed to the highest dose of approximately 40 mg/kg/d (360 mg/l)**.
- In the main study,
    - **decreased colloid in F0 males exposed to the highest dose of approx. 300 mg/kg/d (3000 mg/l),**
    - **increased TSH in male F1 pups at PND28 at the intermediate dose of approx. 40 mg/kg (360 mg/L) and**
    - **increased thyroid weight and decreased T4 in female F2 pups at PND21 at the intermediate dose of approx. 237 mg/kg/d (1000 mg/l).**

A high individual variability was observed in the measure of thyroid hormones. Blood collection for hormone analysis was not standardised and was performed at various times of the day (8:45 AM to 5:30 PM) whereas circadian variations were described for TH (Jordan *et al*., 1980; Ahlersova *et al.,* 1997). Additional variability may have impaired the capacity of the study to detect significant changes, in particular for TSH that has a short half-life.

A number of changes were not statistically significant but were observed with noticeable variations (>10% compared to controls) and with a p value < 0.3. With the exception of an increase in T4 in F1 pups at PND4 in the preliminary study, they were consistent with the pattern of the statistically significant changes observed i.e. increased TSH, increased T3 and decreased T4 levels.

By inhalation, resorcinol induced thyroid hyperplasia in 39% of animals (none in controls) when administered at 220 ppm for 90 days, without significant change of thyroid weight.

A difference in results between gavage and administration through a physiological oral route such as diet or drinking water is noted and these differences are not explained by the level of doses administered.

Because of the effective conjugation of resorcinol by hepatic enzymes (Eilstein *et al*., 2020), a contribution of **sublingual absorption that bypasses the first pass metabolisation in the gastrointestinal tract may lead to a slower metabolisation and hence higher systemic exposure to free active resorcinol when the substance is administered in diet or drinking water compared to gavage**. The importance of the contribution of sublingual absorption has been demonstrated with other substances such as bisphenol A (Gayrard *et al*., 2013; Vandenberg *et al*., 2014). Another difference between modes of administration is that gavage results in a bolus dose whereas other modes of administration result in a more prolonged exposure and it may have an impact on the development of effects. However, the thyroid is generally poorly investigated in gavage studies and effects via this route of exposure may also be underdetected.

The role of iodine supply has also been considered. NRC's Fourth Revised Edition of Nutrient Requirements of Laboratory Animals (1995) sets iodine requirement in diet for rats at 0.15 mg/kg. This value was unchanged since the 1978 edition. Seffner *et al*. (1995) refers to requirements of 0.1 to 0.2 mg/kg iodine in diet. Bianco *et al*. (2014) considers that the chow diet fed to rodents in accredited animal facilities generally contains enough iodine (0.4–1 mg/kg) to allow for a normal daily iodine intake and thyroid function, while a level as low as 0.02 mg I/kg) can promote iodine deficiency.

For resorcinol, in studies performed by oral route, no data on iodine content in the diet is available for Berthezene (1979) and a low-iodine diet is reported in Cooksey (1985)

without further information. Data can be found for all other oral studies and are in the range 0.3 to 3.37 mg/kg iodine in diet, i.e. within the range of recommended requirements, in all other studies. Iodine content in the diet is not known for the older publications and in particular for all studies performed by dermal and subcutaneous route, which prevents further discussion of the influence of iodine intake on the results.

In Cooksey *et al*. (1985), the animals were fed with a low-iodine and low-protein diet. Importantly, treated animals were compared to controls fed with the same diet. Therefore, the diet cannot be responsible for the effects observed in resorcinol-treated animals. It is also unknown whether the diet iodine content was actually lower than 0.15 mg/kg feed, a dose considered sufficient for rats. If it happened that the diet contained less than 0.15 mg/kg, this might have potentiated the negative effect of resorcinol as compared with a normal diet. However, this would not invalidate the relevance of this study. Indeed, low-iodine diet is unfortunately prevalent in the general population as well. For example, the study by Steinmaus *et al*. (2016) performed in South California reports that a large proportion of the women (74%) had urinary iodine levels below the recommended median level (150 µg/L) for pregnancy. Rayman & Bath (2015) reports that the United Kingdom is now classified as mildly iodine deficient by the World Health Organization, based on a 2011 National study of 14-15-year-old schoolgirls. In addition, it is well established that pregnancy, a critical stage for thyroid-dependant neurodevelopment, is associated with about a 30% increase in iodine requirements (200 vs 150 µg I/day for pregnancy and adults, respectively; Williams textbook of endocrinology, 2016). According to medical surveys, maternal iodine supply is very often insufficient to cover pregnancy requirements (Glinoer *et al*., 1990). Thus studies conducted with mild iodine deficiency can be considered as relevant to evaluate the impact of thyroid disrupters. On the contrary, in animal studies using high dietary iodine content (as seen in the NTP studies), the thyroid might be less sensitive to resorcinol since iodide attenuated the inhibition of TPO by Resorcinol (Divi & Doerge (1994)). In addition, for any study on thyroid disruption, it is important to keep in mind that iodine itself has regulatory function in the thyroid. For example, iodine plays a role in goiter formation independent of TSH (Gerard *et al*., 2008), and iodide deficiency renders the thyroid more sensitive towards TSH (see the review by Dumont *et al*., 1992).

An effect on adrenals was also observed in several studies. A significant effect on the adrenal weight is observed in the NTP 90-day studies, in both rats and mice, in absence of any thyroid effect. Histopathologic findings in the adrenals (large vacuolated cells) were also reported, together with thyroid effects, in the older study of Samuel (1955) by dermal and subcutaneous route. An effect on adrenal weight was also noted in females in the 90-day inhalation study. Given the relationship between thyroid and adrenals (Wondisford, 2015), it cannot be excluded that effects on adrenals may be related to disruption of TH, although this relationship has not been studied in details in the scientific literature.

Table 25: Summary of findings in studies investigating thyroid

Reference	ToxR-tool	Species	Vehicle	Duration	Doses (M/F)	Parameter measured						
						Thyroid weight	Histo	TSH	T3	T4	MIT/DIT	I uptake
SUBCUTANEOUS												
Klein 1950	3	Rabbits	Saline	4 or 15 d	50 or 75 mg/kg/d	=	=					
Cheymol 1951	3	Wistar rats	Aqueous solution	Every 2 d for 1 mo.	50 mg/kg/d	(+)	(+)					
Arnott & Doniach 1952	2	Rats	Water or ethanol: water	single	42 to 180 mg/kg							↘
Doniach & L. 1953	2	Rats	Oil	10 to 69 days	308 mg/kg/d	+	+					
			Water	Single	55 mg/kg							-
Samuel 1955	3	Wistar rats	Peanut oil	21 to 38 d	308 to 396 mg/kg/d	+	+					
			Beeswax in peanut oil	21 to 79 d	792 mg/kg/d	+	+					
DERMAL												
Doniach & L. 1953	2	Rats	Ointment	3 wk	NA	=						
Samuel 1955	3	Wistar rats	Ointment	28 d	8 000 mg/kg/d	+	+					
GAVAGE												
NTP 1992	1	B6C3F1 mice	Water	90 days	28, 56, 112, 225 or 420 mg/kg/d		=					
				2 years	112 or 225 mg/kg/d		=					
		F344 rats		90 days	32, 65, 130, 260 or 520 mg/kg/d		=		=	=		
				2 years	50, 112/100 or 225/150 mg/kg/d		=					
USR, 2004a	1	SD rats	Water	90 days	40, 80 or 250 mg/kg/d	F: ↘	=					

DIET OR DRINKING WATER												
Berthezene 1979	3	Rats	Diet	14 days	2 000-2 500 mg/kg	↗				↘	↗	
Cooksey 1985	2	Wistar rats	DW	30 days	9.9 mg/kg/d	↗			↘ T3+T4			
Seffner 1995	2	Rats	DW	84 days	2.56-2.92 mg/kg/d		+					
USR, 2003	1	SD rats	DW	F0: approx. 81 days	0.8/0.8, 3.9/5.1, 13.1/15.6 or 36.9/46.6 mg/kg/d	=	M/F: (+)	M:(↗)	F:↗	=		
				F1: GD0- PND4				=	(↗)	(↗)		
				F1: GD0- PND28	5.0, 18.5, 58.7 or 174.4 mg/kg/d	=		M:(↗)	F:(↗)	=		
USR, 2005a (Welsch, 2008a)	1	SD rats	DW	F0: approx. 133 days	11/17, 33/51, 88/123 or 246/294 mg/kg/d	=	M:+	M:(↗)	M:(↗)	=		
				F1 pups: GD0- PND28	31, 98, 245 or 674 mg/kg/d	=	=	M:↗	=	=		
				F1 adults: GD0 to approx. PND133	14/18, 41/48, 115/141 or 304/347 mg/kg/d	=	=	=	=	=		
				F2 pups: GD0- PND21	28, 85, 237 or 645 mg/kg/d	F: ↗ M:(↗)		=	=	F:↘ M:(↘)		
INHALATION												
USR, 1977	3	SD rats	-	90 days	220 ppm	=	+					

USR: unpublished study report; NA: data not available; F: females; M: males; DW: drinking water
= : no effect observed
+ : effect observed (not significant but >10% and p<0.3 when into brackets)
↗: increase observed (not significant but >10% and p<0.3 when into brackets)
↘: decrease observed (not significant but >10% and p<0.3 when into brackets)
Cell left empty when parameter not investigated

Neurobehavioural effects

Neurobehavioural effects are investigated only in two studies.

In the 90-day study performed by gavage in 2004, motor activity was unaffected but an increase in the response in the landing foot splay test was observed in females exposed to ≥ 80 mg/kg. The landing foot splay test detects sensorimotor dysfunctions. In case of sensorimotor dysfunction, the imprints of the hind feet are larger.

In relation to developmental neurobehavioural effects, a FOB was conducted in F1 pups exposed *in utero* and thereafter in the preliminary reproductive toxicity study with administration of test substance in drinking water (2003). An increased locomotor activity was determined in males at sexual maturation (PND60). No effect was observed on acoustic startle response and spatial memory. However, the results obtained for spatial memory should have been corrected in relation to this increased motor activity since it can influence performance of the animals in the Biel maze swimming test. For instance, an increased motor activity may compensate a slight memory deficit. In this study, the modifications of locomotor activity were not considered while constituting a serious bias impeding a correct evaluation of spatial memory.

Considering the thyroid effects of resorcinol and effects on motor activity in the preliminary study, it is unfortunate that developmental neurobehavioural parameters were not included in the main reproductive toxicity study that would have allowed to characterise these effects with a higher number of animals.

Clinical signs indicative of neurotoxicity are observed in acute toxicity studies by various routes of exposure and at different levels of dose depending on the route of exposure (WHO, 2006). After repeated administration, neurotoxic signs are reported at 50 mg/kg/d in one study by subcutaneous route (Cheymol *et al*., 1951) and in the studies performed by gavage. In the NTP studies conducted by gavage (17-day, 90-day and 2-year studies), clinical signs including ataxia, prostration, salivation, recumbency and tremors were seen in treated male and female rats and mice. These clinical signs of toxicity began shortly after chemical administration, lasted from 30 minutes to an hour, and became more pronounced at the end of each 5-day dosing period. In rats, it was observed at the highest dose of 520 mg/kg in both sexes in the 90-day study and from 100 mg/kg in the 2-year study. The LOAEL for neurotoxic signs was in the 17-day range finding study, with hyperexcitability observed in female rats dosed at and greater than 55 mg/kg bw by gavage.

Similarly, the majority of rats given 250 mg/kg/day in the 90-day study performed by gavage (Unpublished study report, 2004) experienced intermittent convulsive movements and excessive salivation from around week 7 until the end of the dosing period. These clinical signs are suggestive of an acute effect of resorcinol on the central nervous system. The mode of action of this acute neurotoxicity is unknown.

In the 90-day study by gavage, an increase in landing footsplay was observed in females from 80 mg/kg/d in a test performed after week 10. This indication of a sensorimotor dysfunction therefore occurs at a dose below those inducing acute neurotoxic effects although measurement of landing footsplay could be more sensitive than visual check for clinical signs.
Finally, no clinical signs indicative of acute neurotoxicity are reported in the preliminary and main two-generation studies conducted in drinking water. In this study, an increased locomotor activity was observed in males at sexual maturation (PND60) and was statistically significant from 40 mg/l (2.8-18.5 mg/kg/d), i.e. at doses well below those where acute neurotoxicity is described in other studies here above. Besides, F1 rats selected for behavioural testing were not exposed to resorcinol after weaning. This increased locomotor activity can therefore not be attributed to direct neurotoxicity of

Resorcinol on the CNS but to more subtle systemic effects. TH disruption is a biologically plausible explanation. There is no data available to support other modes of action.

Other developmental effects

Prenatal toxicity studies were all conducted by gavage. No significant effect was reported in rabbits. No significant effect was observed on foetal body weight in rats. In Sprague-Dawley rats, a non-significant but dose-related increase in skeletal variations, in particular incomplete ossification of parietals and interparietals was observed at doses without significant maternal toxicity (Unpublished study report, 1982a). This observation was repeated in a further study (Unpublished study report, 2004b) with a statistically significant increase on a foetal basis but without dose responses. Maternal toxicity, absent in these studies, cannot explain these results. The consistency of these observations supports that resorcinol may alter craniofacial growth in rats. It is also noted that all developmental studies were conducted by gavage and therefore may have lacked sensitivity.

4.4.3. Conclusion on the adverse effect of resorcinol on thyroid function

The effects of resorcinol on thyroid are established in humans based on a series of ten human cases with severe clinical hypothyroidism. Reversal of symptoms and of goitre after cessation of the exposure to resorcinol demonstrate that resorcinol is the determining factor.

The presence of resorcinol in drinking water is also suspected to be responsible for the locally high incidence of goiters in children in western Columbia.

In animals, effects on thyroid were observed after exposure by subcutaneous, dermal, inhalation as well as oral (diet or drinking water) routes. The effects observed in the different studies are consistent with each other and echo the thyroid effects demonstrated in humans. Based on a weight of evidence, the pattern of effects in animals consists of increased thyroid weight, **histological findings (depletion of colloid, hyperplasia), high TSH, high T3 and reduction of T4**.

A difference in the severity of the effects is observed between human and experimental data. The discrepancy is not fully understood but it can be partly explained by differences in routes of exposure as further discussed in section 6.3.2.1.

ECHA/EFSA guidance (2018) on identification of ED in Biocides and Plant Protection Products states that "Using the current understanding of thyroid physiology and toxicology (European Commission, 2017), it is proposed that the following be applied when interpreting data from experimental animals:

1) Substances inducing **histopathological changes** (i.e. follicular cell hypertrophy and/or hyperplasia and/or neoplasia) in the thyroid, with or without changes in the circulating levels of THs, would **pose a hazard for human thyroid hormone insufficiency** in adults as well as pre- and post-natal neurological development of offspring.
2) Substances that **alter the circulating levels of T3 and/or T4 without histopathological findings would still present a potential concern for neurodevelopment**."

A modification of thyroid histology is considered as a sensitive and early endpoint to demonstrate thyroid disruption (Bianco *et al*., 2014). As it reflects an attempt to compensate for insufficient levels of TH, it is considered as a reliable indicator of repeated TH disturbance.

Thyroid hyperplasia was observed in several studies (Doniach & Logothetopoulos, 1953; Samuel, 1955; F0 animals in the preliminary reproductive toxicity study, unpublished study report, 2003; 90-day study by inhalation, unpublished study report, 1977). T3 and/or T4 were also significantly altered in several studies (Berthezene 1979, Cooksey *et al.,* 1985; F2 pups in the main reproductive toxicity study, unpublished study report, 2005). Therefore, induction of histological modifications in the thyroid and alteration of the concentration of T4 by resorcinol in experimental studies are adverse effects.

Developmental effects of resorcinol on cognitive function are poorly investigated. No human data are available. It was assessed only experimentally in the preliminary study of the two-generation study through a FOB. Effects on locomotor activity were observed in males (unpublished study report, 2003).

Thyroid hormones play an important role in the foetal development and more particularly in prenatal brain development. The impact of decreased T4 during brain development is highlighted in two Adverse Outcome Pathways (AOP) that have been recently validated and published by the OECD in 2019:

- "Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals" (Crofton *et al*., 2019; AOPWiki 42)
- "Inhibition of Na+/Isymporter (NIS) leads to learning and memory impairment" (Rolaki *et al*., 2019; AOPWiki 54).

By different molecular initiating events, both AOP lead to a decrease in T4 with neurodevelopmental consequences. In both AOP, **the relationship between a decrease in T4 serum concentration and a decrease in cognitive function is considered to be established with a high level of weight of evidence and with high relevance during foetal and perinatal stages during brain development.**

Considering these AOP and the capacity of resorcinol to alter T4 levels in humans and experimental animals, neurobehavioural effects of resorcinol are therefore probable.

Finally, indications of skeletal variations showing retardation of skull ossification were observed in rats after developmental exposure to resorcinol (unpublished study report 1982a, unpublished study report 2004b).

4.5 Endocrine mode of action

4.5.1. *In vitro* information indicative of endocrine activity of resorcinol in relation to thyroid

In vitro information related to the regulation of thyroid hormones are summarised below.

Effect of resorcinol on TPO inhibition, iodine uptake and organification

Fawcett & Kirkwood (1953) (ToxRtool score 3) tested resorcinol for its capacity to decrease the incorporation of labeled iodine into the organically-bound form in thyroid slices from two female albino rats. The degree of inhibition was evaluated by comparing the radioautographs of the chromatograms from hydrolysate extracts of exposed tissue and control tissues. The minimal concentration that produced an anti-thyroid effect is 0.2 10^{-6} M. The authors reported that resorcinol reacts instantaneously with iodine in aqueous

solution. They suggested formation of a compound stably bound to iodine and identified 4-iodoresorcinol in the treated thyroid tissue.

Coval & Taurog (1967) (ToxRtool score 3) measured activity of TPO purified from porcine thyroid tissue in presence of $^{131}I^-$ and used bovine serum albumin as iodide acceptor. Radioactivity incorporation into MIT, DIT and T4 was measured by scintillation counting after chromatography. **Addition of resorcinol resulted in a markedly reduced iodination activity to 1.5% of controls at 5 µM and 0.93% of controls at 50 µM resorcinol.**

Taurog (1970) (ToxRtool score 2) used a similar protocol but with a more highly purified TPO from porcine tissue (5 times greater specific activity) and compared results to either oxidation of gaiacol, followed by the change in absorbance at 470 nm, or oxidation of iodide (I^-) into I_3^-. **An half inhibitory concentration (IC_{50}) of 0.71 µM was reported when measuring** bovine serum albumin **(BSA) iodination, 0.17 µM for guaiacol oxidation and 0.45 µM for I_3^- formation. In all test systems, resorcinol was more potent than MMI.**

Berthezene *et al.* (1979) (ToxRtool score 3) incubated thyroid lobes (species not mentioned) with radioiodine and resorcinol concentration 10^{-6} to 10^{-3} M. An increase of thyroid radioiodide, indicative of an increased thyroid uptake was observed as well as an increase of MIT/DIT ratio (2.2±0.2 vs 1.0±0.1 in control, $p<0.001$).

TPO inhibition was investigated in two *in vitro* experiments (Cooksey *et al.* (1985), ToxRtool score 3; Lindsay *et al*., 1992, ToxRtool score 2).

A first experiment was conducted to measure porcine thyroid peroxidase-catalysed iodination of BSA. TPO was extracted and purified from hog thyroids. The activity of thyroid peroxidase was calculated after separation and determination of ^{125}I-BSA from unbound ^{125}I after incubation with resorcinol for 10 min at 37°C with purified porcine TPO. Twelve experiments were conducted in duplicate with four concentrations tested in each experiment. PTU and MMI were used as reference standards. **IC_{50} for resorcinol was 0.27±0.01 µM (mean±standard error). It was 26.7 times more potent than PTU (IC_{50}=7.2 µM) and 15.5 times more potent that MMI (IC_{50}=7.2 µM)**.

When tested separately, a TPO inhibition of 34% and 27% was observed with 0.2 µM resorcinol and 0.2 µM 2-methylresorcinol, respectively. Inhibition was 73% with a combination of 0.2 µM of each compound. Similar results were obtained for a combination of resorcinol with 5-methylresorcinol. A synergistic effect with other dihydroxyphenols was therefore demonstrated. Additive effects were observed for a combination of resorcinol and thiocyanate.
In a second experiment, ^{125}I uptake and ^{125}I incorporation into MIT, DIT, T3 and T4 on porcine thyroid slices were measured at 0.2 (1 experiment) and 0.5 µM (6 experiments) after 4 hr incubation at 37°C. MMI (3 concentrations, 12 experiments/conc.), PTU (2 concentrations, 3 or 4 experiments/conc.) and potassium thiocyanate (2 concentrations, 1 experiment/conc.) were used as positive controls. Each experiment was performed in duplicate. **^{125}I uptake was 79.7% of control at 0.2 µM (not significant) and 78.5% of control at 0.5 µM ($p\leq0.05$).** The effect was comparable to the effect of 12 µM PTU (77.3% of inhibition, $p\leq0.05$) and 1.8 µM MMI (77.3%, $p\leq0.05$) or 10 µM potassium thiocyanate (78.6%, not significant). Cooksey *et al.* (1985) investigated additional concentrations and reported that inhibition was stronger at concentrations 0.2-0.5 µM than 10-50 µM. **^{125}I organification into MIT, DIT, T3 and T4 was significantly decreased at 0.5 µM (-97.3%, $p\leq0.05$).** The effect (-22.4%) was not significant at 0.2 µM. Resorcinol was more potent than MMI (85% inhibition at 1.8 µM) and PTU (93.6% at 12 µM).

Divi & Doerge (1994) (ToxRtool score: 3) incubated resorcinol with porcine TPO, or lactoperoxidase (LPO) that were used as a model peroxidase involving *in vitro* iodination. TPO and LPO were incubated at 25°C in phosphate buffer with resorcinol (8.0 µM/250 µM) in the presence or absence of hydrogen peroxide (substrate for iodide organification), iodide or pyrogallol (alternate substrate). Measurements were performed at least in duplicate. Enzyme activity was quantified spectrophotometrically after 2 min of incubation by measuring absorbance at 289 nm due to MIT. **Incubation of resorcinol with LPO or TPO in the presence of hydrogen peroxide resulted in a time-dependent loss of enzymatic activity.** LPO activity in the presence of hydrogen peroxide and 250 µM ^{14}C-resorcinol was reduced to 17.3±6.6% of control. Addition of 0.1 mM of iodide ion (I^-) modestly enhanced LPO inactivation (6.2±3.3% of control) but a **higher concentration of 5.0 mM I^- reduced LPO inactivation (44.7±4.5% of control)**. Inhibition of 1.0 µM LPO with 200 µM resorcinol was 98% and of 0.1 µM TPO with 25 µM resorcinol was 97%. Maximal rate of inactivation of LPO (k_i) was 1.77 min^{-1} and apparent binding constant (K_i) 3.48 µM. Incubation of ^{14}C-resorcinol with LPO resulted in a covalent binding of radioactivity that could not be dissociated by dialysis or chromocentrifugation. The presence of iodide ion (0.1 or 5.0 mM) did not prevent covalent binding whereas the presence of pyrogallol (≥1.0 mM) prevented the binding almost completely. **Inactivation of LPO by resorcinol was pH-dependent and inactivation rate increased as pH decreases.** At 250 µM resorcinol, remaining activity was 18.2±5.4% for LPO (1 µM) and 12.9±3.4% for TPO (0.1 µM). The activity of other peroxidases (myeloperoxidase, chloroperoxidase, horseradish peroxidase and ferric myoglobin) was not decreased below 95%. The authors concluded that the data point toward a peroxidase-mediated oxidation of resorcinol producing a reactive species that covalently binds at or near the haeme-containing active site to irreversibly block enzymatic activity.

In a poorly reported abstract, Gaitan *et al.* (1995) (ToxRtool score: 3) measured uptake of ^{125}I in rat thyroid cell line FRTL-5. Resorcinol (50 µM) further increased ^{125}I uptake in presence of TSH (8.2±1.0 pmol/µg DNA vs 6.6±0.9 pmol/µg DNA for TSH alone). Addition of ouabain (1 mM) partially suppressed the uptake (4.1±0.4 pmol/µg DNA), indicating that the effect of resorcinol is mediated by Na^+-K^+-ATPase.

Resorcinol (purity specification ≥99;0%, purity not further confirmed analytically by US EPA, personal communication from the author) was included in a 21-chemical training set in the development of a high-throughput screening TPO inhibition assay by US EPA (Paul *et al*., 2014) (ToxRtool score 1). A fluorescent peroxidase substrate, Amplex UltraRed (AUR) was converted to Amplex UltroxRed in presence of hydrogen peroxide. To detect TPO inhibition, AUR was added on microplates with rat thyroid microsomal proteins from male Long Evans rats, with the test compound and hydrogen peroxide. Fluorescence was measured after 30 min. 15 concentrations of each chemical in DMSO were tested (range: 0.053 nM to 253 µM) and 3 biological replicates performed. The experimental conditions of the assay were optimised by preliminary tests using MMI and by comparison to the existing guaiacol oxidation assay that measure spectrophotometrically TPO-mediated oxidation of guaiacol to yellowish-brown diguaiacol using rat thyroid microsomes. **Resorcinol gave a positive response to TPO inhibition with a low potency.** The lowest test concentration that yielded a 20% decrease of TPO activity was 253 µM, the highest concentration tested. **Predicted IC_{50} for resorcinol was 253 µM and relative IC_{50} 317 µM. Relative potency was $1x10^{-4}$ (IC_{50}=0.031 µM) compared to MMI and $4x10^{-4}$ compared to PTU (IC_{50}=0.12 µM).**

Resorcinol was used as one of the 10 model compounds (5 TPO-inhibitors and 5 with a non-TPO mode of action on thyroid) to validate a luminometric assay for the detection of inhibition of human TPO (Jomaa *et al.*, 2015) (ToxRtool score 1). Human TPO was derived from the human thyroid follicular cell line Nthy-ori 3-1, a cell line in which TPO relative gene expression has been previously quantified by others. The effect on the activity of the human TPO was quantified by measuring the oxidation of luminol in the presence of hydrogen peroxide, which resulted in emission of light at 428 nm. The same assay was

also conducted with TPO purified from porcine thyroid tissues. Model compounds were also tested with the AUR-TPO assay using TPO from human cell line. Inhibition was also compared to results obtained using gaiacol substrate on porcine TPO, determined experimentally or obtained from literature e.g. for resorcinol (Taurog, 1970). For each assay, three independent experiments were conducted with four well replicates in each experiment. Five concentrations were tested (approx. range 10^{-2} to 10^{3} µM).

All model compounds were adequately detected in all assays. Sensitivity of the assay was higher than the gaiacol assay**. IC_{50} of resorcinol was 5.0 µM (CI: 3.6-6.9) on human TPO and 5.8 µM (CI: 4.0-8.3) on porcine TPO in the luminol assay. IC_{50} was 18.7 µM (CI: 6.8-51.9) on human TPO in the AUR assay.** In the gaiacol assay a value of 6.5 on porcine TPO was reported in Taurog (1970). **In all assays, potency ranking was as follows: MMI>resorcinol>PTU.**

Resorcinol (purity specification ≥98,5%, determined to be 93.95% by US EPA's analysis) was also included in a larger tiered high-throughput screening approach to identify TPO inhibitors developed by US EPA (Paul Friedman *et al*., 2016) (ToxRtool score 1). 1074 chemicals including resorcinol were tested at a single concentration of 87.5 µM in the AUR-TPO assay using rat microsomes as described above by Paul *et al.* (2014). Resorcinol induced 81.3% of inhibition of TPO **activity** and was further tested in the AUR-TPO assay at 6 concentrations (range: 0.0854 to 87.5 µM). **An IC_{20} of 0.006 µM and an IC_{50} of 0.025 µM were determined for resorcinol. The maximum observed inhibition was 81.8%.** Based on IC_{50}, resorcinol was 2.4 times more potent than MMI (IC_{50}=0.06 µM) and 9 times more potent than PTU (IC_{50}=0.23 µM). A firefly luciferase inhibition assay (Quanti-Lum) was used to evaluate nonspecific enzyme inhibition in a cell-free model, and a cytotoxicity assay using a human cell line (HEK293T) was employed over a similar concentration range to estimate the cellular concentration limit to identify reactive compounds. No significant cytotoxicity was observed on human cell line. Luciferase inhibition > 20% was observed from 5.47 µM. A selectivity of 2.84 was calculated and **resorcinol was considered as a highly selective TPO-inhibitor** (selectivity >1). The marked potency differences observed in TPO inhibition for resorcinol in this study and in the previous study (Paul *et al*., 2014) was noted by the study's authors and a difference in test chemical quality or purity was suggested.

<u>Other tests related to thyroid</u>

Takemura *et al.* (1966) (ToxRtool score 2) investigated the effect of resorcinol on the uptake of radiolabeled T4 in muscle tissue from adult male Wistar rats in the presence of rat plasma. *In vitro*, 1 µM resorcinol resulted in a decrease of T4 uptake by the muscle to 71% of control ($p<0.01$) during a 2-hr incubation. It was considered by the authors that this result reflected an effect of resorcinol on plasma protein-T4 interaction (increase). Indeed, it is assumed that the availability of T4 to the cells depends upon the relative binding capacity of the plasma and perhaps the specific binding protein for T4 in the cells. However, other interpretations can be plausible. In the light of more recent understanding of the role of active transporter of TH in the cells, the effect observed may also indicate competitive inhibition of TH transporters by resorcinol.

Resorcinol was tested for its capacity to inhibit TSH-stimulated cAMP production in Chinese hamster ovary cells transfected with the recombinant human TSH receptor (Santini *et al.*, 2003) (ToxRtool score 2). No inhibition of TSH-induced AMPc production was observed after incubation of cells for 1 hour at 37°C with 1000 µM resorcinol in 95% ethanol in presence of bovine TSH.

Thyroid hormone-like activity of resorcinol was tested in the T-screen assay (Ghisari & Bonefeld-Jorgensen, 2009) (ToxRtool score 2) using rat pituitary tumour cell line GH3 expressing intracellular TH and ER receptors and responding to physiological concentrations of TH by proliferation. A concentration range of $1x10^{-10}$ to $1x10^{-5}$ M

resorcinol in DMSO was tested in the absence or in the presence of T3 at its EC_{50} concentration. At least three independent experiments were conducted. **Resorcinol stimulated proliferation of the GH3 cells at the highest concentration tested (50 µM), in the absence or in the presence of T3** ($p<0.05$).

Resorcinol was included in the EU-funded ENDOMET project[14] and was tested in 23 different assays related to endocrine metabolism and function (Waring *et al.*, 2012) (ToxRtool score 3). In relation to thyroid function, a T-screen assay was conducted on rat pituitary GH3 cell line in absence and in presence of T3. The rat thyroid PCCL3 cell line was used to measure human NIS gene expression in presence of resorcinol, without and with T3 at its EC_{50} concentration. Transcriptional activity of NIS thyroid promoter construct and cytotoxicity to rat thyroid FRTL cells were also measured. Results of other assays are summarised in the section below. **Resorcinol induced a TH-dependent proliferative effect in the T-screen assay at 10 µM. No effect was detected on gene expression or transcription of NIS or on cytotoxicity to FRTL cells**.

Table 26: Summary of *in vitro* data investigating thyroid function

Method	ToxRtool	Results	Reference
Inhibition of TPO			
Measure of the incorporation of labeled iodine into the organically bound form in thyroid slices from two female Albino rats.	ToxRtool 3 Minor limitations: ▪ absence of information on purity of test substance ▪ test concentrations not presented Major limitations: ▪ no information on number of replicates ▪ method of determination of inhibition not fully explained Used in a WoE approach as supporting study	Minimal concentration that produced an anti-thyroid effect was 0.2 10^{-6} M 4-iodoresorcinol identified to be formed in the treated thyroid tissue.	Fawcett & Kirkwood (1953)
Measure of inhibition of porcine purified TPO using BSA as iodide acceptor.	ToxRtool 3 Minor limitations: ▪ absence of information on purity and source of test substance Major limitations: ▪ design poorly presented (no information on method of determination of inhibition not fully explained ▪ limited reporting of results Used in a WoE approach as supporting study	Iodination activity decreased to 1.5% of controls at 5 µM and 0.93% of controls at 50 µM.	Coval & Taurog, 1967

[14] The ENDOMET project (funded by the EC 5th Framework programme) was set up to examine the relative contributions of genomic and non-genomic pathways to the endocrine disrupting effects of a range of plasticisers and environmental pollutants.

Measure of inhibition of porcine purified TPO using BSA or guaiacol as test system.	ToxRtool 2 Minor limitations: ▪ absence of information on purity and source of test substance and number of replicates	BSA assay: IC50 = 0.71 µM (MMI: 7.9 µM) Guaiacol assay: IC50 = 0.17 µM (MMI: 0.23 µM) I3- assay: IC50 = 0.45 µM (MMI: 0.23 µM)	Taurog 1970
Measure of inhibition of enzymatic activity of porcine purified TPO on iodination of BSA. 12 experiments with 4 concentrations performed in duplicate	ToxRtool 2 Minor limitations: ▪ absence of information on purity of test substance and source of test system	IC_{50} = 0.3 µM Higher potency than PTU (x26.7) and MMI (x15.5)	Lindsay *et al*. 1992 Cooksey *et al.* 1985 (ToxRtool 3)
Measure of activity of purified porcine TPO and LPO by measure of MIT formed from iodination of tyrosine	ToxRtool 3 Minor limitations: ▪ absence of information on purity of test substance and source of test system Major limitations: ▪ absence of positive controls ▪ confused presentation of design and results Used in a WoE approach as supporting study	Time-dependent loss of enzymatic activity of LPO or TPO. Covalent binding of ^{14}C-resorcinol with LPO. Reduction of LPO activity to 17.3±6.6% of control with 250 µM ^{14}C-resorcinol. 98% inhibition of LPO with 200 µM resorcinol and 97% of TPO with 25 µM resorcinol LPO*:* k_i=1.77 min^{-1} ; K_i=3.48 µM Remaining activity with 250 µM resorcinol: 18.2±5.4% for LPO (1 µM) and 12.9±3.4% for TPO (0.1 µM).	Divi & Doerge, 1994
AUR-TPO: Measure of fluorescence after incubation of Amplex Ultrared (fluorescent peroxidase substrate) and rat microsomal proteins 15 concentrations in DMSO (range: 0.053 nM to 253 µM) ; 3 biological replicates for each	ToxRtool 1	IC_{50} = 253 µM Relative potency $1x10^{-4}$ compared to MMI and $4x10^{-4}$ compared to PTU.	Paul *et al*. 2014
Luminase assay: measure of luminescence after incubation of luminol and TPO from human thyroid cell line or porcine thyroid tissues. AUR-TPO: measure of fluorescence after incubation of Amplex Ultrared (fluorescent peroxidase substrate) and	ToxRtool 1	<u>Luminol assay:</u> IC_{50} human TPO = 5.0 µM (CI: 3.6-6.9) IC_{50} porcine TPO = 5.8 µM (CI: 4.0-8.3) <u>AUR-TPO assay:</u> IC_{50} human TPO = 18.7 µM (CI: 6.8-51.9) Potency ranking: MMI>resorcinol>PTU	Jomaa *et al*. 2015

TPO from human thyroid cell line 5 concentrations in DMSO; 3 independent experiments with 4 replicates each			
AUR-TPO: Measure of fluorescence after incubation of Amplex Ultrared (fluorescent peroxidase substrate) and rat microsomal proteins 6 concentrations in DMSO (range: 0.0854 to 87.5 µM) ; 3 biological replicates for each + luciferase inhibition assay (Quanti-Lum) + cytotoxicity assay on human cell line (HEK293T) to detect nonspecific enzyme inhibition	ToxRtool 1	IC_{20} = 0.006 µM IC_{50} = 0.025 µM 2.4 more potent than MMI (IC_{50}=0.06 µM) and 9 times more potent than PTU (IC_{50}=0.23 µM) Maximum observed inhibition: 81.8% Identified as a highly selective inhibitor	Paul Friedman *et al*. 2016.
Iodine uptake			
Measure of radioiodine uptake from thyroid lobes (species not mentioned) Concentrations from 10^{-6} to 10^{-3} M	ToxRtool 3 Major limitations: ▪ experimental conditions poorly described (absence of information on purity of test substance, lack of positive and negative controls, details of experimental protocol, etc), ▪ limited reporting of results. Used in a WoE approach as supporting study	Increase in thyroid radioiodide concentration and in MIT/DIT ratio (2.2±0.2 vs 1.0±0.1 in control, p<0.001) was observed.	Berthezene *et al.* 1979
Measure of ^{125}I uptake from porcine thyroid slices	ToxRtool 2 Minor limitations: ▪ absence of information on purity of test substance and source of test system	Significant inhibition of uptake at 0.5 µM (78.5% of control), comparable to PTU at 12 µM and MMI at 1.8 µM Inhibition stronger at concentrations 0.2-0.5 µM than 10-50 µM.	Lindsay *et al*. 1992 (ToxRtool 2) Cooksey *et al*. 1985 (ToxRtool 3)
Uptake of ^{125}I in rat thyroid cell line FRTL-5	ToxRtool 3 Major limitations: ▪ very poor description of experimental conditions and reporting of results (abstract). Used in a WoE approach as supporting study	^{125}I uptake (pmol/µg DNA): Negative control: 2.8±0.2 TSH: 6.6±0.9 Resorcinol + TSH: 8.2±1.0 Resorcinol + TSH + ouabain: 4.1±0.4	Gaitan *et al*. 1995 (ToxRtool 3)

Iodine organification			
Measure of incorporation of ^{125}I into MIT and DIT from porcine thyroid slices 6 concentrations from 0.2 to 50 µM	ToxRtool 3 Minor limitations: ▪ absence of information on purity of test substance and source of test system Major limitations: ▪ limited description of conditions of the tests and results Used in a WoE approach as supporting study	Marked increase in ratio I/(MIT+DIT) from 0.5 µM indicating inhibition of incorporation	Cooksey *et al.* 1985
Measure of incorporation of ^{125}I into MIT, DIT, T3 and T4 from porcine thyroid slices 2 concentrations : 0.2 (1 exp.) and 0.5 µM (6 exp.)	ToxRtool 2 Minor limitations: ▪ absence of information on purity of test substance and source of test system	Inhibition of organification by -91.3 % at 0.5 µM (not significant at 0.2 µM)	Lindsay *et al*. 1992
Effect on NIS			
Measure of human NIS gene expression, without and with T3 at its EC_{50} concentration and measure of transcriptional activity of NIS thyroid promoter construct in rat thyroid PCCL3 cell line	ToxRtool 3 Minor limitations: ▪ absence of information on purity of test substance and source of test system Major limitations: ▪ limited description of conditions of the tests (including test concentrations) and no numerical results presented Used in a WoE approach as supporting study	No effect detected	Waring *et al.* 2012
Cellular T4 uptake			
Uptake of radiolabeled T4 in muscle tissue from adult male Wistar rats in the presence of rat plasma Concentration: 1 µM	ToxRtool 2 Minor limitations: ▪ absence of information on purity and source of test substance ▪ no information on number of replicates, method for statistical analysis	Decreased T4 uptake to 71% of control ($p<0.01$)	Takemura *et al.* 1966
Inhibition of TSH receptor			
Inhibition of TSH-stimulated cAMP production in Chinese hamster ovary cells transfected with the	ToxRtool 2 Minor limitations: ▪ absence of information on purity and on number of replicates,	No effect	Santini *et al*. 2003

recombinant human TSH receptor Concentration: 1 to 1000 µM	▪ no numerical result presented		
Induction of TH-dependent cell proliferation			
T-screen assay: measure of TH-dependent proliferation of rat pituitary tumour cell line GH3. Concentration range: $1x10^{-10}$ to $1x10^{-5}$ M in DMSO	ToxRtool 2 Minor limitations: ▪ not all quantitative results presented	Increased proliferation ($p<0.05$) at the highest concentration tested (50 µM), in absence or in presence of T3.	Ghisari *et al*. 2009
T-screen assay: measure of TH-dependent proliferation of rat pituitary tumour cell line GH3.	ToxRtool 3 Minor limitations: ▪ absence of information on purity of test substance and source of test system Major limitations: ▪ limited description of conditions of the tests (including test concentrations) and no numerical results presented Used in a WoE approach as supporting study	Measurable effect at 10 µM	Waring *et al.* 2012

Exp.: experiment

Information related to other hormonal systems and biological activities

Studies investigating a potential activity of resorcinol on endocrine systems other than thyroid detected no estrogenic or anti-estrogenic activity of resorcinol. Antagonist activities to AhR and AR were detected (Krueger *et al*., 2008). A number of ED activities were screened in Waring *et al*. (2012) and a positive response was observed only for the inhibition of the aromatase activity. In the US EPA Toxcast program, resorcinol was found active in 6 assays related to endocrine disruption and inhibition of TPO (as reported in more detail in Paul Friedman *et al.,* 2016) was the most sensitive target of resorcinol.

These results are presented in more detail in Annex I and only shortly summarised here as these alternative modes of action are not considered relevant to explain the effects of resorcinol on thyroid observed in humans and experimental animals.

Summary of *in vitro* data

Several studies investigated the effects of resorcinol on TPO, using either TPO purified from porcine thyroid tissues or human thyroid cell lines or rat thyroid microsomes and using different substrates (tyrosine, guaiacol, BSA, fluorescent Amplex Ultrared, luminol). **Inhibition of TPO was consistently identified in these studies independently of the test system**. Differences in potency were reported across studies. The lowest potency was measured in rat microsomes (IC_{50}=253 µM, Paul *et al.,* 2014) whereas a subsequent study using an identical protocol determined a much higher potency (IC_{50}=0.025 µM, Paul Friedman *et al.,* 2016). A difference in purity of resorcinol was mentioned by authors as a possible explanation of the discrepancy in the results but may hardly explain the strong

variability in values.

Intermediate values of potency were reported by Lindsay *et al.* (1992) (IC_{50}=0.3 µM) and Taurog (1970) (IC_{50} ranging from 0.17 to 0.71 µM) in pig and by Jomaa *et al*. (2015) (IC_{50} ranging from 5 to 18.7 µM) in rat and human TPO.

It is unlikely that the differences in potency reflect differences in species sensitivity. E.g. potencies obtained in rat cells represent the smallest and highest values. In a further experiment that investigated comparative porcine and rat thyroid microsomal TPO activity in response to a subset of 12 chemicals not including resorcinol, Paul *et al*. (2014) showed that results were qualitatively concordant and yield similar relative potency values, suggesting similar sensitivity of TPO in the rats and pigs. In addition, **in one study investigating the effect of resorcinol on TPO from a human thyroid cell line, the potency of resorcinol was found to be similar in human and porcine TPO (Jomaa *et al*., 2015).**

Although the differences in potency across studies may reflect the heterogeneity of experimental conditions as well as individual variability, they remain partly unexplained.

The level of potency of resorcinol can be approached by comparison with known potent TPO inhibitors such as PTU and MMI that have been used in humans as drugs for this property. These known TPO inhibitors are used as positive controls in several studies and confirm the sensitivity and adequacy of the corresponding test systems.

Table 27: Ranking of resorcinol potency compared to known potent TPO inhibitors MMI and PTU

Reference	Ranking (IC_{50} in µM)
Taurog 1970[a]	**Resorcinol** (0.75) > MMI (7.9)
Lindsay *et al*., 1992	**Resorcinol** (0.27) > MMI (4.2) > PTU (7.2)
Paul *et al*., 2014	MMI (0.025) > PTU (0.12) > **resorcinol** (253)
Jomaa *et al*., 2015[b]	MMI (4.0) > **resorcinol** (5.0) > PTU (16.4)
Friedman *et al*., 2016	**Resorcinol** (0.025) > MMI (0.06) > PTU (0.23)

[a] Results provided on BSA iodination but same ranking with other assays of the study
[b] Results provided on luminol assay with human cell line but same ranking with other assays of the study

With the exception of Paul *et al.* (2014) that reported an exceptionally low potency for resorcinol, resorcinol has a higher or intermediate potency compared to MMI and PTU in all other studies. **Resorcinol can therefore be considered as a potent TPO inhibitor *in vitro* compared to drugs with known effects in humans.**

Results from Divi & Doerge (1994) indicated that inhibition of TPO by resorcinol is influenced by iodine concentration in the media, with a higher concentration of iodine resulting in reduced inactivation.
No direct effect of resorcinol on the NIS transporter was reported (Waring *et al.*, 2012) but contradictory results were reported on the effect of resorcinol on iodine uptake in thyroid explants.

Consistently with TPO inhibition, resorcinol was observed to inhibit the incorporation of iodine into thyroid hormones and its precursors using porcine thyroid tissues (Berthezene *et al*., 1979, Cooksey *et al*., 1985, Lindsay *et al*., 1992).

Resorcinol did not alter AMPc-dependent signalling pathways of TSH (Santini *et al.*, 2003) but resorcinol showed *in vitro* an agonist effect to thyroid hormones in two T-screen assays from 10 µM (Ghisari *et al*., 2009, Waring *et al.,* 2012).

4.5.2. Analysis of the endocrine mode of action

In vitro data provides **evidence that resorcinol is a potent TPO inhibitor** compared to PTU and MMI in cells from porcine, rat or human origin independently of the test system used.

TPO is a key enzyme of thyroid hormone synthesis**. Consequences of TPO inhibition on decreased TH synthesis and decreased T4 levels in serum are described in the OECD AOP dedicated to TPO inhibition** (Crofton *et al*., 2019). These key event relationships (KER) are considered established with a high level of evidence although their quantitative understanding is low to moderate (Fig. 3).

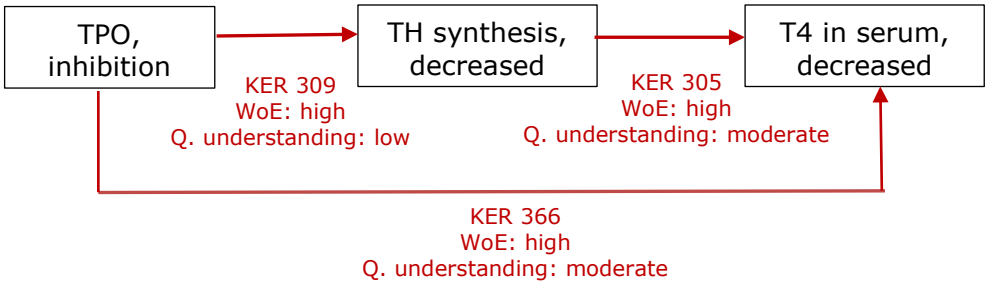


Figure 3: Key events relationships of the endocrine mode of action of TPO inhibition from AOP 42[15] (OECD AOP n°13, Crofton *et al*., 2009) (Q=quantitative)

Therefore, inhibition of TPO activity is widely accepted to directly impact TH synthesis and this is supported by more than three decades of research in humans and animals (Cooper *et al.,* 1982; Cooper *et al*., 1983; Divi and Doerge, 1994).

For resorcinol, the consequences of TPO inhibition on thyroid hormones are reported in some experimental studies as well as in human case reports in which it was investigated.

In vitro, in porcine thyroid slices, resorcinol is associated with strong inhibition (-97.3%) of organification of iodine at concentrations of 0.5 µM (Cooksey *et al*., 1985, Lindsay *et al*., 1992) whereas there is no direct effect on NIS. MIT/DIT ratio was increased (Berthezene *et al*., 1979).

In vivo in experimental animals, a significant decrease of T4 was observed in Berthezene *et al.* (1979) at high doses (2000 to 2500 mg/kg/d) in diet for 2 weeks as well as in female F2 pups exposed to approx. 237 mg/kg/d at PND21 in drinking water in the reproductive toxicity study (Unpublished study report, 2005a). A decrease that is not statistically significant but > 10% and with p<0.3 is observed in F2 male pups (-19%, p=0.184). In contrast, an increase in T4 was observed in F1 pups in the preliminary study (+34%, p=0.053) at PND4. The significance of this increase is uncertain. The thyroid system is very active during the early phase at PND4 and until the stabilisation of the hypothalamic-pituitary-thyroid (HPT) axis around PND 14-15. In addition, the presence of resorcinol and the level of exposure of pups through maternal milk is not known. The increase might result from compensatory mechanisms after *in utero* deficiency. No solid conclusion can be drawn from this finding but it illustrates the complexity of thyroid regulation.

An increase in intrathyroid MIT / DIT ratio and a decrease in thyroid iodine uptake were also observed after dietary administration of 10 mg/kg/d for 5 days (Berthezene *et al.,* 1979).

[15] https://aopwiki.org/aops/42

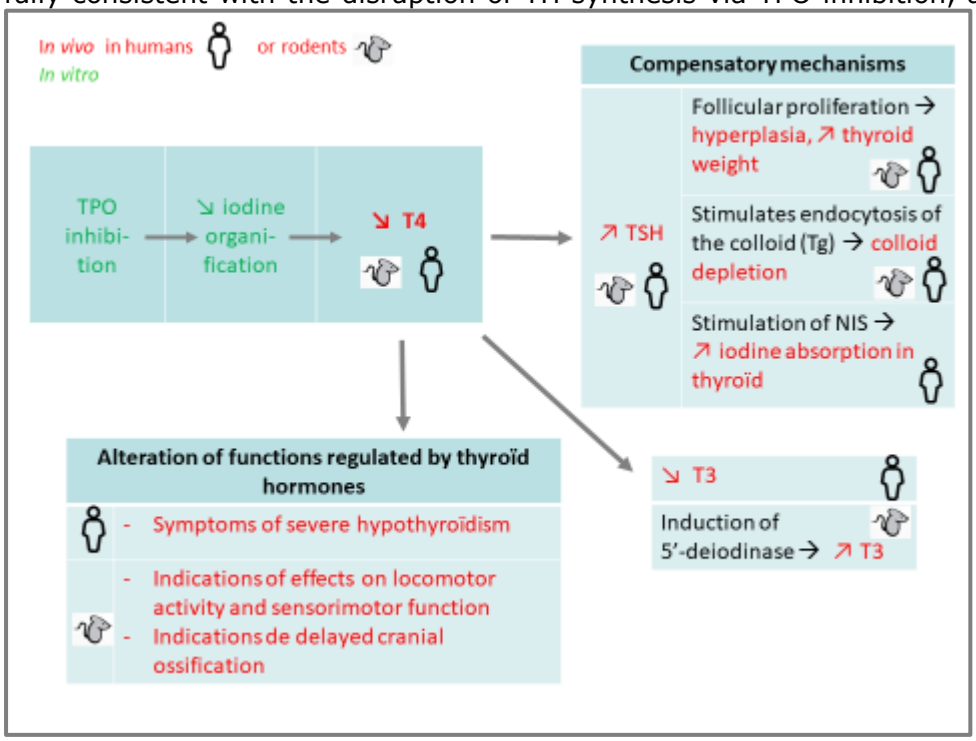
In humans, low concentrations of thyroid hormones in the blood were reported in several human cases with a low PBI (Bull & Fraser, 1950; Hobson, 1951), and low T4 and/or T3 (Berthezene *et al*., 1973; Katin *et al*., 1977).

Therefore, based on a weight of evidence, there is strong evidence that resorcinol acts via an endocrine MoA via the inhibition of the key enzyme of TH biosynthesis TPO and with consequences observed in humans and in animals on the blood concentrations of thyroid hormones, in particular a decrease in T4.

4.6 Plausible link between adverse effects and endocrine mode of action

In humans, the adverse effects that are observed are consistent with the blockage of thyroid hormone synthesis.

The adverse effects reported in experimental animals appear milder. However, they are fully consistent with the disruption of TH synthesis via TPO inhibition, as summarised in



below.

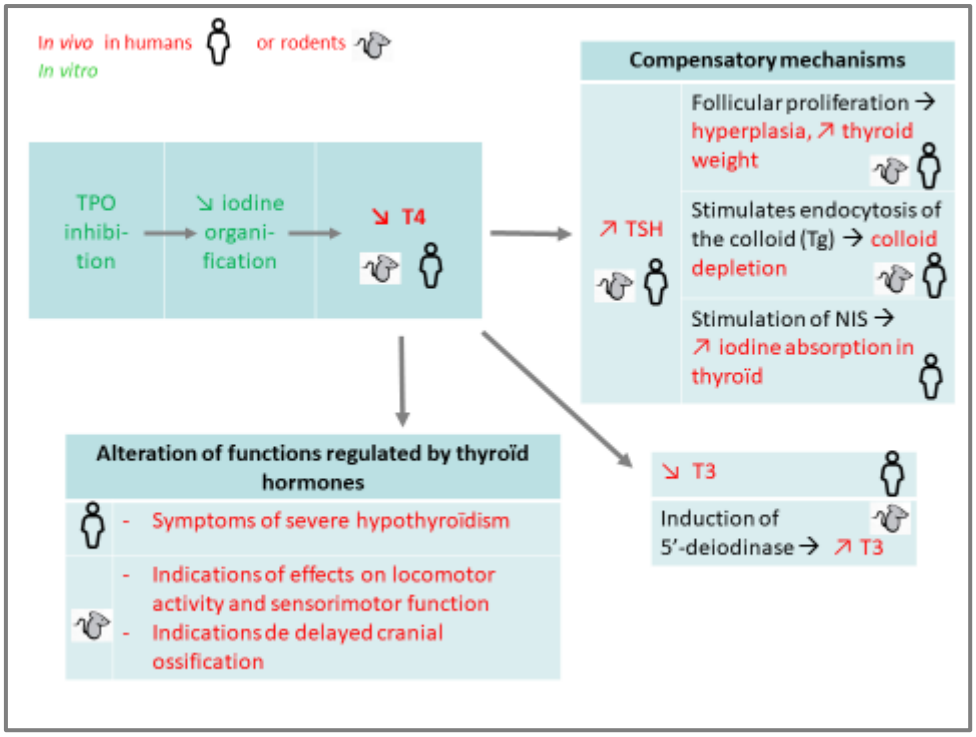


Figure 4: Biological plausible link between findings observed after exposure to resorcinol

Decreased T4 is a direct consequence of inhibition of TH synthesis resulting from TPO inhibition. Modification of the MIT/DIT ratio also indicates that the organification process leading to TH synthesis is disturbed.

Compensatory mechanisms in response to the increase in TSH due to the partial suppression of the negative feedback of thyroid hormones are not able to restore a normal thyroid function because of the direct inhibition of TPO. Colloid depletion observed in rats and in human cases is consistent with increased Tg endocytosis in response to TSH increase. In humans, in the absence of a direct effect on NIS as observed in *in vitro* data presented above (section 4.5.1), the increase in iodine uptake may be related to an enhanced expression of NIS by TSH.

Considering the type of effects observed in humans and in experimental animals, they are generally consistent with each other and with the mode of action identified. Diverging observations are however reported for T3. In humans, T3 is measured in one case (Katin *et al*., 1977) and a decrease is observed, compatible with severe inhibition of organification in the long term. In rats, an increase in T3 is noted. About 80% of circulating T3 originates from hepatic deiodination of T4. The concentration of T3 in the serum therefore does not only reflect the T3 synthesis rate in the thyroid. Induction of deiodinase and in particular deiodinase-2 have been observed in response to hypothyroidism (Peeters & Visser, 2017). The observation of an increase in T3 may therefore result from the activation of deiodinase-2 in response to a decrease in T4.

A similar pathophysiological scheme has been established in human cases with a TPO mutation resulting in a moderate residual activity (around 30%) of TPO (Narumi *et al.,* 2017). Patients were not identified at birth in TSH-based screening for congenital hypothyroidism but presented goitre at ages of 8 to 19 years. Evaluation of the thyroid function showed mild elevation of serum TSH levels, normal or slightly low serum T4 levels, high serum T3 to T4 molar ratio, high serum thyroglobulin levels and high thyroidal ^{123}I uptake. These observations are in line with the physiopathological pattern of effects observed in experimental animals exposed to resorcinol and indicate that a moderate inhibition of TPO can result in goitre in the long-term in humans.

An increase an increase in locomotor activity is observed in males exposed both *in utero* and after. The nervous system, in particular during its phase of development, is a sensitive target of thyroid homeostasis disruption. The relationship between TPO inhibition, the decrease in T4 levels in serum and alteration of cognitive function is established with a strong level of evidence in the OECD AOP related to TPO inhibition (Fig. 5 below, Crofton *et al*., 2019).

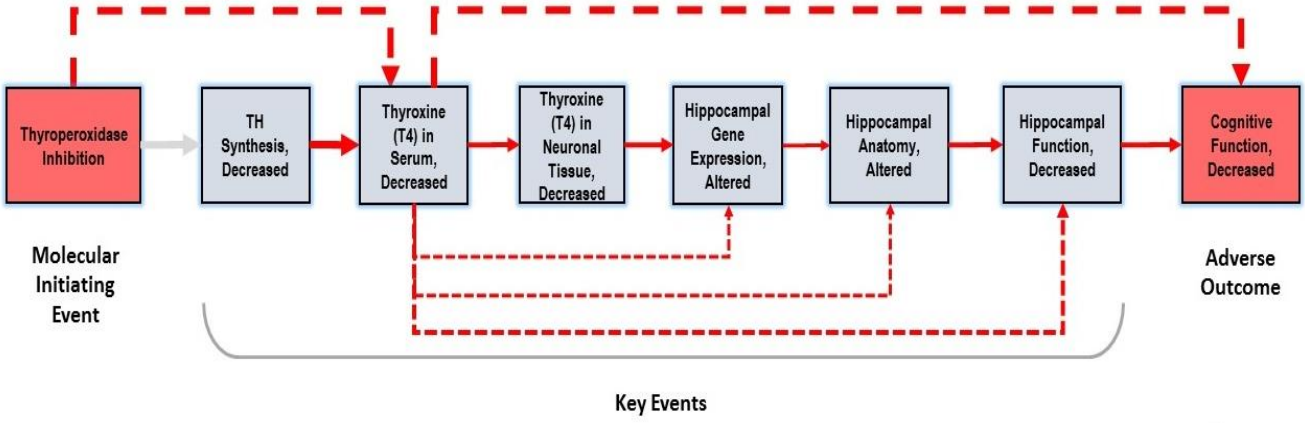


Figure 5: AOP Inhibition of Thyroperoxidase and Subsequent Adverse Neurodevelopmental Outcomes in Mammals

Changes in thyroid hormones or other hormones/factors during the early developmental period could trigger long-term modifications in the neural structures underlying locomotor activity, thereby resulting in changed activity during adulthood (Zoeller & Rovet, 2004). Eisenbrandt *et al*. (1994) and ECETOC (1992) have concluded that "*Interpretation of results from motor activity tests is difficult, especially in terms of direct v. indirect effects on the nervous system. A dose-related change in motor activity reflects an effect on the nervous system only in the absence of general toxicity;*" In the case of resorcinol, no general toxicity is observed and the observed effects may be related to thyroid disruption by resorcinol.

Indications of craniofacial variations are observed in the two rat prenatal studies by gavage. Skeletal variations are generally not considered of sufficient adversity to trigger a developmental classification according to CLP and can be specific or unspecific manifestations of many toxic effects including maternal toxicity. Maternal toxicity was however absent in the rat prenatal studies and cannot explain these findings. The skeleton is extremely sensitive to thyroid hormones, which have profound effects on bone development, linear growth and adult bone maintenance (Bassett & Williams 2016). Thyroid hormones are important for skull bone growth, which primarily occurs at the cranial sutures and synchondroses. Thyroid hormones regulate metabolism and act in all stages of cartilage and bone development, differentiation and maintenance by interacting with growth hormone and regulating insulin-like growth factor 1. An excess in thyroxine has been shown to be associated with craniofacial growth disorders both in humans and mice (Howie *et al*., 2016; Durham *et al*., 2017; Kesterke *et al*., 2018). A recent publication (Leitch *et al.* 2020) describes that "Thyroid hormone deficiency causes delayed craniofacial and tooth development, dysplastic facial features and delayed development of the ossicles in the middle ear. Thyroid hormone excess, by contrast, accelerates development of the skull and, in severe cases, might lead to craniosynostosis with neurological sequelae and facial hypoplasia." Although they are not specific of such an MoA, the indications of skeletal variations observed in rats may possibly be related to resorcinol and its thyroid-disrupting MoA.

Based on current understanding of endocrinology and physiology, the biological plausibility that adverse effects observed in experimental animals and humans exposed to resorcinol can be linked to its known endocrine MoA via TPO inhibition is high.

Table 28: Summary of human and experimental data providing evidence of an effect of resorcinol on the thyroid and contributing to the weight of evidence (reliability score)

	In vitro	Experimental data *in vivo*	Human data
TPO inhibition	Observed in : Studies with reliability score of 1 or 2: • Taurog 1970 (R2) • Lindsay 1992 (R2) • Paul 2014 (R1) • Jomaa 2015 (R1) • Paul Friedman 2016 (R1) Supporting studies with reliability score of 3: • Divi & Doerge 1994 (R3)		
Decreased organification	Observed in : Studies with reliability score of 1 or 2: • Fawcett & Kirkwood 1953 (R3) • Lindsay 1992 (R2) Supporting studies with reliability score of 3: • Coval & Taurog 1970 (R3) •	Observed in : Studies with reliability score of 1 or 2: • Cooksey 1985 (R2) Supporting studies with reliability score of 3: • Berthezene 1979 (R3)	
T4		Decreased T4 in: Studies with reliability score of 1 or 2: • Cooksey 1985 (T3+T4) (R2) • Main two-generation study (Unpub. 2005a) (R1) Supporting studies with reliability score of 3: • Berthezene 1979 (R3)	Indications of low TH in: • Bull & Fraser 1950 (low PBI) • Hobson 1951 (low PBI) • Berthezene 1973 (low T4) • Katin 1977 (low T4 and T3)
T3		Elevated T3 in: Studies with reliability score of 1 or 2: • Preliminary two-generation study (Unpub. 2003) (R1)	
TSH		Increased TSH in: Studies with reliability score of 1 or 2: • Main two-generation study (Unpub. 2005a) (R1)	High TSH level in Katin *et al.* 1977
Histological findings in thyroid		Observed in: Studies with reliability score of 1 or 2: • Doniach 1953 (R2) • Seffner 1995 (R2) • Main two-generation study (Unpub. 2005a) (R1) Supporting studies with reliability score of 3: • Cheymol 1951 (R3) • Samuel 1955 (R3)	Observed in: • Bull & Fraser 1950

		• 90-day inhalation study (Unpub. 1977) (R3)	
Increased thyroid weight/thyroid enlargment		Observed in: Studies with reliability score of 1 or 2: • Doniach 1953 (R2) • Cooksey 1985 (R2) • Main two-generation study (Unpub. 2005a) (R1) • Supporting studies with reliability score of 3:Samuel 1955 (R3) • Berthezene 1979 (R3) •	Goitre in all human cases
Consequences to TH alteration		Studies with reliability score of 1 or 2: • Increased landing footsplay in 90-day study (Unpub. 2004) (R1) and increased motor activity in the preliminary two-generation study (Unpub. 2003) (R1) • Indication of skeletal craniofacial variations in two prenatal rat studies (Unpub. 1982a (R1), Unpub. 2004b (R1))	Clinical signs consistent with severe hypothyroidism in all human cases

4.7 Human relevance

The adverse effects of resorcinol on thyroid function as well as its property to disrupt thyroid hormone synthesis have been established in humans. *In vitro,* inhibition of TPO by resorcinol has been confirmed in human thyroid cell lines in Jomaa *et al.* (2015).

Effects have been also observed in experimental animals using non artificial routes of exposure that are widely accepted as adequate to investigate and characterise effects relevant for humans. The pattern of effects that is observed in animals is consistent with, but of lower severity than, similar effects reported in human cases as discussed in the previous section.

The relevance of the effects and ED properties of resorcinol for humans is therefore unequivocally established.

4.8 Conclusion regarding ED properties relevant for human health

It is well established based on a series of case reports, where high doses of resorcinol were given, that severe hypothyroidism was diagnosed and reversed when exposure to resorcinol was stopped. This leads to the conclusion that exposure to resorcinol can affect the regulation of the thyroid function inducing hypothyroidism in humans. The endocrine disrupting properties of resorcinol have therefore been demonstrated in humans under these specific conditions of exposure.

The medical cases reporting these effects are patients that applied resorcinol to damaged skin for 9 out of 10 cases. However, considering that the skin was reported as intact in one additional case and that skin absorption has been demonstrated *in vitro*, these data are considered relevant in the assessment.

In addition, findings consistent with the MoA (mode of action) of thyroid disruption via TPO (thyroperoxidase) inhibition are also reported in several experimental studies via drinking water. Similar findings reported in studies conducted by subcutaneous, dietary and inhalation routes provide supportive evidence. In particular histopathological changes in the thyroid and changes in the circulating levels of T3 (triiodothyronine) or T4 (thyroxine), are considered as adverse effects.

Inhibition of TPO by resorcinol, a key enzyme in the synthesis of thyroid hormones, is established *in vitro* in several experimental designs.

Altogether, the effects observed in humans and in some experimental studies are fully consistent with the MoA via TPO inhibition. Based on current knowledge, the biological plausibility of a causal link between inhibition of TPO, disruption of thyroid hormone levels and adverse effects linked to low thyroid hormones levels is strong. A recently validated AOP (adverse outcome pathway) described the relationship between inhibition of TPO, decreased T4 and neurodevelopmental alteration due to maternal low T4 concentration as having a high level of evidence for humans (AOP n°42). With the validation of this AOP by the OECD (Crofton *et al.,* 2019), further experimental data investigating (neuro)development are not necessary to confirm the effects observed in the preliminary two-generation study available in the registration dossier.

Based on a weight of evidence analysis of the available data, there is scientific evidence that resorcinol can have adverse effects on human health through thyroid disruption and fulfils the definition of an endocrine disruptor.

Table 29: Summary of evidence fulfilling the definition of an endocrine disruptor recommended by JRC (2013)

Adverse effects	Plausible ED MoA	Human relevance
Alteration of thyroid function • Goitre and severe hypothyroidism **in humans** • Decreased T4 and histological thyroid effects in experimental animals; indications of motor dysfunctions; indications of skeletal variations in pups • Probable consequences of maternal hypothyroxynemia on neurodevelopment	**Inhibition of TPO** = key enzyme of TH synthesis • Demonstrated *in vitro* • Supported by findings in experimental and human data consistent with this MoA • Biological plausibility of a causal link highly supported by current knowledge (AOP)	**Effects and MoA identified in humans** • Also supported by *in vitro* data showing TPO inhibition in human cell line • ECHA/EFSA (2018) : humans and rodents considered equally sensitive to thyroid-disruption

5. Endocrine disruption relevant to the environment

The present document focuses on endocrine disrupting properties relevant for human health. However, data on endocrine disruption in vertebrates of the environment exist. They are presented for information to provide a comprehensive description of data, with consideration of the fact that the HPT axis is highly conserved across evolution in vertebrates.

In a short-term screening test zebrafish eleutheroembryos (48 hours post-fertilisation) were exposed for three days to freshly prepared test solutions of one of 25 test substances, including resorcinol, under semi-static conditions (Thienpont *et al*., 2011) (ToxRtool 2).

Thyroid gland functionality was evaluated as a decrease in the intrafollicular T4 content (IT4C). In the first set of the experiment, the IT4C in embryos exposed to resorcinol at the maximum tolerated concentration of 200 mg/L was significantly decreased in comparison to controls ($p < 0.05$) and therefore resorcinol is regarded as a thyroid gland function disruptor and a TPO inhibitor in zebrafish eleuthoembryos. In the second set of the experiment 5 to 8 test substance concentrations were tested for concentration-response curves. EC10 and EC50 values were determined to describe thyroid disrupting potency. The thyroid disrupting index (TDI, LC50/EC50) was used as a descriptor of thyroid disrupting hazard. An EC50 value of 82 ± 37 µM (ca. 9.02 mg/L) and an EC10 value of 2 ± 4 µM (ca. 0.22 mg/L) were reported for thyroid disrupting potency of resorcinol. A NOEC value of 10 µM (1.1 mg/L) was obtained from experimental data by performing one-way ANOVA analysis. Systemic toxicity, expressed as LC50, was 5003 ± 100 µM (ca. 550 mg/L) for resorcinol, resulting in a TDI of 61. Based on a data set of 25 substances, the concentration of intrafollicular T4-content was shown to be sensitive to a direct effect on thyroid gland function, such as TPO inhibition.

In another short-term screening test, zebrafish eleutheroembryos (48h post fertilisation, 30 embryos per concentration and replicate) were exposed for three days to 5 concentrations (and a control) in semi-static conditions (Jarque *et al*., 2018, ToxRTool score 2). The study utilised the transgenic (Tg) zebrafish line Tg(tg:mCherry) in which the reporter gene mCherry (encoding a membrane-bound red fluorescent protein) is under the control of the thyroglobulin (tg) promotor. Therefore, the fluorescent protein mCherry is expressed specifically in the thyroid and is correlated with the expression of thyroglobulin. An increase of fluorescence is indicative of an increase in the synthesis of thyroglobulin in response to increased TSH stimulation of the gland (thyroglobulin is upregulated by TSH) due to the repression of thyroid hormone synthesis by the test substance. At 120 hours post-fertilisation (hpf), embryos were analysed by fluorescence microscope. Induction of fluorescence by resorcinol was observed, with a maximum fold induction of 2.1 in comparison to negative control. At a concentration up to 100 µM, repression of fluorescence or weaker induction of the fluorescence was observed. An EC50 value of 3.4 ± 1.6 µM (ca. 0.37 mg/L) for fluorescence induction and a LC50 value of 5197 µM (ca. 572.2 mg/L) were observed, resulting in a TDI of 1529. For the other TPO inhibitors tested, EC50 ranged from 279 µM for MMI, 366 µM for ethylenethiourea to 1096 µM for phloroglucinol. A BMD20 (concentration at which a 20% increase of the *tg*:mCherry fluorescence was observed) value of 0.663 µM (ca. 0.073 mg/L) was determined for resorcinol. This study therefore provides an indication that resorcinol alters thyroid hormone synthesis *in vivo* in fish.

Taken together as a weight of evidence of the available information, resorcinol presents ED properties impacting the thyroid gland function in fish, especially the thyroid hormones. It is reflected by the decrease of IT4C in zebrafish eleutheroembryos and recently highlighted again by data from a screening study, indicating that resorcinol induces fluorescence in the thyroid gland of zebrafish eleutheroembryos which have been specifically genetically modified for the detection of ED compounds. However, none of the available studies provide information on the apical and adverse effects as a consequence of the capacity of resorcinol to affect thyroid regulation. An AOP related to "Inhibition of thyroid peroxidase leading to impaired fertility in fish"[16] is currently under development on AOPwiki but is not validated yet. The available and current information is therefore not sufficient to draw a final conclusion, i.e. to meet the conditions that define an endocrine disruptor relevant for the environment.

[16] AOP Wiki 271 https://aopwiki.org/aops/271

6. Conclusions on the SVHC Properties

6.1 CMR assessment

Not relevant for the identification of the substance as SVHC in accordance with Article 57 (f) REACH due to its endocrine disrupting properties for human health.

6.2 PBT and vPvB assessment

Not relevant for the identification of the substance as SVHC in accordance with Article 57 (f) REACH due to its endocrine disrupting properties for human health.

6.3 Assessment under Article 57(f)

6.3.1 Summary of the data on the hazardous properties

Resorcinol fulfils the definition of an endocrine disruptor relevant for human health as summarised in section 4.8 above.

6.3.2 Equivalent level of concern assessment

In agreement with the REACH legal text, substances identified as SVHCs under article 57(f) shall give rise to an equivalent level of concern to those of other substances listed in points (a) to (e) and shall be identified on a case-by-case basis.

Human case reports are a major element of the identification of the ED effects of resorcinol. They have been identified under specific conditions and this may raise some questions on their relevance for different conditions of human exposure (Lynch *et al*., 2002; Welsch 2008b). This point is addressed in section 6.3.2.1 as the first element of concern.

In addition, a number of factors relevant to assess that an adverse health effect represents an equivalent level of concern (ELoC) to a CMR substance are identified in a discussion paper by ECHA (2012) with a specific focus on sensitisers. It is however not considered to be a list of requirements that need to all be met to allow the conclusion of an equivalent level of concern that shall be established on a case by case basis. The factors identified in this document to evaluate the ELoC are considered fully relevant for the present case that also relates to SVHC identification according to article 57(f) of REACH for human health. They are listed below and are used in the present analysis:

- Health effects:
  - Type of possible health effects
  - Irreversibility of health effects
  - Delay of health effects
- Other factors:
  - Quality of life affected
  - Societal concern
  - Is derivation of ‘safe concentration’ possible?

6.3.2.1 Relevance of effects in different conditions of human exposure – toxicokinetic considerations

Comparison with reference compounds PTU and MMI

Resorcinol is metabolised quickly in rats and rabbits via the oral route and excreted in urine (90-93% in 24h), mainly as monoglucuronide conjugate (Garton *et al*., 1949; Kim & Matthews 1987). Half-lives of 55 and 22 min respectively are measured *in vitro* in human hepatic S9 fraction and primary human hepatocytes (Eilstein *et al*., 2020). Genies *et al.* (2019a and 2019b) also observe *in vitro* that 23 to 90% of absorbed resorcinol is metabolised locally in the skin after 24h. A total clearance of 139 ml/min was estimated in humans in a PBPK / PD model simulating oral ingestion (Leonard *et al*., 2016).

This rapid clearance is intermediate between clearance of PTU (270 ml/min) and MMI (81 ml/min), drugs with known effects in humans (Leonard *et al*., 2016). Therefore, induction of effects in humans cannot be excluded on the basis of a quick metabolisation. Motonaga *et al*. (2016) report that PTU and MMI have a tendency to accumulate in the thyroid in rats and such a tendency is not observed with resorcinol. Further comparison of the relative potency of resorcinol and PTU and MMI *in vivo* is therefore difficult.

However, if metabolisation of resorcinol is substantial, it is not total. Unmetabolised resorcinol is detected in urine 24h after oral administration (11.4% in Garton & Williams, 1949; 1.2-4.6% in Kim & Matthews, 1987). *In vitro*, 10 to 77% of the absorbed dose in human skin over 24h is parent resorcinol (Genies *et al*., 2019b) and kinetic data indicate that metabolisation is progressive in the skin (no metabolisation after 2h *in vitro*).

A systemic exposure to parent resorcinol by different routes of administration cannot be excluded.

Comparison of effects of resorcinol in humans and in experimental animals

The human cases result from exposure to daily doses of 2 to 140 mg/kg/d on a subchronic to chronic basis. In almost all cases, exposed skin was damaged, which may have contributed to a high systemic availability of unconjugated resorcinol.

Even if these conditions of exposure in humans may be considered exceptional, the effects in the human cases were severe. Resorcinol also induces thyroid effects in experimental studies on rats by different routes of exposure and at different doses but effects are less severe than effects reported in human cases.

Several elements need to be considered when comparing the difference of severity in effects between humans and experimental animals.

- Uncertainties in induction of milder hypothyroidism in humans

In contrast to severe hypothyroidism that presents characteristic symptoms such as goitre in humans, symptoms of milder hypothyroidism such as fatigue, weight gain, dry skin, are not specific. Subclinical hypothyroidism is generally largely under-diagnosed (Andersen, 2019). In addition, there is no epidemiological study of good quality available that investigates the association between thyroid function and exposure to resorcinol. Available human data are inadequate to explore the induction of milder thyroid effects and cannot be used to exclude the possibility for resorcinol to induce effects in conditions of exposure different than those reported.

- Toxicokinetic considerations

Maximised systemic exposure in human cases cannot explain the differences in the severity of effects between human cases and experimental studies. In addition, the relevance of other routes of exposure cannot be excluded.

The application of resorcinol on damaged skin in most (but not all) human cases most probably resulted in a maximisation of dermal absorption as well as a reduction of dermal metabolic capacity, if existing, although internal exposure in human cases have not been documented.

Administration of resorcinol in a lipophilic vehicle in human case reports may also have influenced its kinetics. It may have allowed for a slow and continuous release of resorcinol into the systemic circulation. However, the role of the vehicle on the toxicokinetics of resorcinol has not been investigated experimentally. In subcutaneous studies, both lipophilic and hydrophilic vehicles have been tested. With hydrophilic vehicles, results were negative (Klein 1950, Doniach 1950) or weakly positive results (Cheymol 1951, Doniach 1953) while they were positive with lipophilic vehicles (Doniach, 1953, Samuel 1955). But doses and/or duration of exposure were also much higher with lipophilic vehicle so that the role of the vehicle cannot be identified. In toxicokinetic studies, 98% of elimination within 24h is described after subcutaneous administration in an aqueous vehicle (Merker 1982) but no data is available with lipophilic vehicle. When applied cutaneously in a hair dye formulation, the half-life for urinary excretion is 31h in humans and monkeys (Wolfram & Maibach, 1985). Overall, no clear conclusion can be drawn on the role of each factor on the internal exposure to resorcinol. Besides, lipophilic vehicles as well as other conditions of exposure that may result in continuous exposure, i.e. inhalation, are considered as relevant routes of exposure.

In human cases, the effects were induced by daily doses of approximately 2 to 140 mg/kg/d for 3 months to 13 years. In experimental studies, histological modifications in the thyroid and alteration of the concentration of T4 by resorcinol were observed from 40 mg/kg/d in the preliminary reproductive toxicity study performed in drinking water. Absorption via the oral route is also considered important over 24h. The range of doses is therefore similar but not the severity of effects. Data are however insufficient to understand the differences in kinetics and if they can explain differences in severity between human cases and experimental studies.

The apparent difference in sensitivity between gavage administration and other oral routes such as diet or drinking water that partly bypass the first-pass hepatic metabolisation support the theory that the absorption kinetics and metabolisation of resorcinol are important in the induction and severity of the effects.

The observation of adverse thyroid effects in animals by several routes of exposure relevant for humans, in particular inhalation and oral routes (diet and drinking water), supports the capacity of resorcinol to induce thyroid effects under conditions of exposure different than those in human cases. The possible involvement of resorcinol present in drinking water as a goitrogenic factor in children in western Columbia is also supportive (Gaitan *et al*., 1978 and 1983.

A recent study has investigated environmental and occupational exposure to resorcinol (Porras *et al*., 2018) by biomonitoring total resorcinol concentration in urine. 99% of the urine samples from non-occupationally exposed subjects (n=101) contained a measurable amounts of resorcinol. Resorcinol concentrations among women (mean 84 µg/l, 95th percentile 2072 µg/l) were clearly higher than the respective concentrations among men (mean 35 µg/l, 95th percentile 587 µg/l). The reasons for this difference remain unclear, although it could be speculated that the use of personal care products could be one possible reason. Alternatively, sexual dimorphisms in the metabolic pathways (not

investigated to our knowledge) could contribute to this difference. The urinary level of hairdressers (n=77, 96% of women) occupationally exposed to resorcinol was similar to the general population and there was no general trend for increasing levels after the work shift. A huge variability in the background levels was however observed, with highest values (> general population) in after-holiday samples. In industrial workers exposed to resorcinol, slightly increased levels in samples after the shifts were observed in workers involved in the manufacture of tyres (n=11), adhesives resins (n=5) and glue-laminated timber (n=6). It suggests that industrial occupational exposure to resorcinol results in variations of systemic levels of resorcinol. Although the relationship with known or unknown sources of exposure is not well characterised, this study illustrates that exposure to resorcinol in real circumstances in humans can result in internal exposure.

This element has to be considered in the light of the low systemic concentrations of free resorcinol that were measured in experimental studies, even though effects were observed (unpublished study reports, 2004a and 2005). **The possibility that resorcinol may induce effects in humans under common conditions of exposure cannot be excluded on a toxico-kinetic basis.**

In particular, the conclusion of Leonard *et al.* (2016) that resorcinol is a substance of very low concern, based on a physiologically-based pharmacokinetic model integrated with pharmacodynamics model (PBPK/PD) simulating oral ingestion in humans, is flawed.

The model was based on the IC_{50} for TPO inhibition reported by Paul *et al.* (2014), which represents the highest value available to characterise the potency of resorcinol on TPO (IC_{50}=253 µM). As discussed in section 4.5.4, a high variability partly linked to the test model used (e.g. organotypic culture vs microsomal fractions) was observed in the results of the studies assessing the potency of resorcinol on TPO inhibition. A later study by the same team and with the same experimental design reported an IC_{50} of 0.025 µM (Paul Friedman *et al.,* 2016). With the exception of these two extreme high and low IC_{50} values, results in other studies range from 0.17 to 18.7 µM. The model of Leonard *et al.* (2016) is therefore most probably largely underestimating resorcinol potency.

- Duration of exposure

It is probable that exposure duration in experimental studies are insufficient compared to the duration of exposure in human cases that are generally longer. With time, the transient resistance to thyroid disruption due to compensatory mechanisms is exceeded. In particular, the development of a goitre requires prolonged thyroid disruption. In patients with moderate residual TPO activity (30%), a goitre was detected only between ages 8 to 19 (Narumi *et al*., 2017).

Even though a long duration of exposure is needed for goitre induction, thyroid disruption limited in time can however have adverse consequences if it takes place during critical periods of development of the central nervous system *in utero*. Shorter conditions of exposure are therefore relevant for the induction of neurodevelopmental adverse effects in humans.

- Sensitive populations

It cannot be excluded that humans or some humans are more sensitive than rats to the effects of resorcinol. Although it is likely that only a minority of existing cases have been published, the relatively small number of human cases published compared to the potential large use of resorcinol-containing ointment in the past may point toward differences in sensitivity within the population. The highest sensitivity can result from the genetic variability of TPO, from latent pre-existing hypothyroidism, an iodine-difficient diet or altered metabolic capacities. The existence of sensitive populations as well as sensitive periods of exposure is further discussed in section 6.3.2.6 related to uncertainties

surrounding dose-reponse for thyroid effects of resorcinol.

The effects induced by resorcinol in humans and in experimental animals are complementary in the demonstration that they cannot be considered as specific to exceptional conditions of exposure, in particular because some populations and periods of exposure can be associated with specific sensitivity.

6.3.2.2 Type of possible health effects and seriousness of the effects

Hypothyroidism

Resorcinol has been directly demonstrated to have the capacity to induce hypothyroidism in humans, via its endocrine disruptive MoA on thyroid hormone synthesis.

Hypothyroidism is a disorder of the thyroid gland that does not produce enough thyroid hormone and is one of the most frequent endocrine disorders. Thyroid hormones are crucial regulators of many physiological functions such as metabolism, homeostasis of the metabolic rate (de Coster & van Larebeke, 2012), bone maintenance (Bassett & Wiliams 2016) and cardiovascular, nervous, immune as well as reproductive functions (Choksi *et al*., 2003).

The most common symptoms of hypothyroidism in adults are fatigue, lethargy, cold intolerance, moderate weight gain, constipation, change in voice, and dry skin but the clinical presentation can include a wide variety of symptoms that range from life threatening to no signs or symptoms depending on age, sex, and time between onset and diagnosis (Chaker *et al*., 2017, Kortenkamp *et al*., 2012). Hypothyroidism has clinical implications related to nearly all major organs. Clinical presentation of overt hypothyroidism is presented in Table 30.

Table 30: Clinical presentation and implications of overt hypothyroidism (from Chaker *et al.,* 2017)

	Presentation	**Signs and implications**
General metabolism	Weight gain, cold intolerance, fatigue	Increase in body-mass index, low metabolic rate, hypothermia* Dyslipidaemia
Cardiovascular	Fatigue on exertion, shortness of breath	Bradycardia, hypertension, endothelial dysfunction or increased intima-media thickness*, diastolic dysfunction*, pericardial effusion*, hyperhomocysteinemia*, electrocardiogram changes*
Neurosensory	Hoarseness of voice, decreased taste, vision, or hearing	Neuropathy, cochlear dysfunction, decreased olfactory and gustatory sensitivity
Neurological and psychiatric	Impaired memory, paresthesia, mood impairment	Impaired cognitive function, delayed relaxation of tendon reflexes, depression*, dementia*, ataxia*, Carpal tunnel syndrome and other nerve entrapment syndromes*, myxedema coma*
Gastrointestinal	Constipation	Reduced oesophageal motility, non-alcoholic fatty liver disease*, ascites (very rare)
Endocrinological	Infertility and	Goitre, glucose metabolism

	subfertility, menstrual disturbance, galactorrhoea	dysregulation, infertility, sexual dysfunction, increased prolactin, pituitary hyperplasia*
Musculoskeletal	Muscle weakness, muscle cramps, arthralgia	Creatine phosphokinase elevation, Hoffman's syndrome*, osteoporotic fracture* (most probably caused by overtreatment)
Haemostasis and haematological	Bleeding, fatigue	Mild anaemia, acquired von Willebrand disease*, decreased protein C and S*, increased red cell distribution width*, increased mean platelet volume*
Skin and hair	Dry skin, hair loss Puffiness	Coarse skin, loss of lateral eyebrows*, yellow palms of the hand*, alopecia areata*, myxedema
Electrolytes and kidney function	Deterioration of kidney function	Decreased estimated glomerular filtration rate, hyponatraemia*

* Uncommon presentation

However, severe hypothyroidism, leading to myxedema, is clearly rare compared to 50 years ago. Indeed, nowadays, hypothyroidism is mostly diagnosed based on biological screening as the clinical spectrum includes non-specific signs. It is considered that the intensity of this subclinical presentation is dependent on the degree of hormone deficiency. Larsen & Davies (2003) described the following manifestations that can be observed in the clinical setting feature:

- Skin is pale, cool and tends to be thinner and finely wrinkled;
- Cardiac output at rest is decreased, perivascular resistance at rest is increased and blood volume is reduced leading to decreased blood flow in tissues, electrocardiographic changes are also observed;
- Modest gain in weight by retention of fluids and reduced appetite; peristaltic activity is decreased and is responsible for frequent complaints of constipation;
- Slow intellectual functions including speech, memory defects; headaches are frequent; depressive mood; body movements are slow and clumsy; paresthesia; hearing loss;
- Stiffness and aching of muscles, slowness of movements;
- Decreased bone formation and resorption;
- Urine flow is reduced and delay in the excretion of water may result in reversal of the normal diurnal pattern of urine excretion;
- Red blood cell mass is decreased; bleeding tendency sometimes occurs;
- Metabolism of androgens and estrogens is altered with possible consequences on reproductive function;
- Decrease in energy metabolism and heat production leading to slightly low basal temperature; insulin response to glucose is delayed; cholesterol, triglycerides and Low Density Lipoproteins (LDL) are increased; prevalence of aortic atherosclerosis and myocardial infarction is increased.

It is therefore a serious condition and the capacity for resorcinol to induce or contribute to existing hypothyroidism raises significant concern.

- **Developmental consequences of decreased level of maternal TH**

High sensitivity of foetus to deficiency in TH

Thyroid hormones play an important role in foetal development and a particularly key role in prenatal brain development as summarised in Figure 6. They control the proliferation of neural progenitor cells, their asymmetric cell division, migration and differentiation into

neurons. In humans, the foetal thyroid gland is not formed until the 2nd trimester of pregnancy. Because the foetal thyroid gland does not start TH synthesis until the third trimester of pregnancy, the developing foetal brain has to rely on TH supply from the mother. TH, specifically, T4, readily crosses the placenta and **the developing foetus is completely reliant on this maternal source of TH during the first half of pregnancy**. Moreover, early life stages may be more sensitive to thyroid-disrupting chemicals, given reduced compensatory abilities and the potential for permanent alterations related to HPT function (Beekhuijzen *et al*., 2019, Gilbert *et al*., 2012).

Figure 6: Role of thyroid hormones in foetal neurologic development in relation to timing of several landmark stages of development in humans. (From Colborn (2004), adapted from Howdeshell (2002)). The figure was deleted due to potential copyright reasons.

The review by Gilbert *et al.* (2012) reports that both maternal hypothyroidism (MH) during pregnancy and congenital hypothyroidism (CH) are associated with reduced IQ levels as well as cognitive and motor deficits. In MH, the type of cognitive deficit reflects the stage of development when mothers' TH levels were low. In CH, deficits in visual and visuospatial domains are reported to reflect gestational TH loss, whereas sensorimotor and language deficits reflect the postnatal duration of TH insufficiency. Notably, both MH and CH groups show memory problems suggestive of compromised hippocampal development, although the groups differ somewhat as to their specific memory deficits. Román *et al.* (2013) also report an association between maternal hypothyroidism and an increased incidence of autistic troubles in 6-year old children and Mughal *et al.* (2018) have emphasised in their review that maternal thyroid hormone signalling during early pregnancy affects not only offspring IQ, but also neurodevelopmental disease risk.

LaFranchi *et al.* (2005) also report that women with subclinical hypothyroidism appear to be at increased risk for some of the pregnancy complications, in particular preterm birth and pregnancy-induced hypertension in some studies.

Overall, hypothyroidism has clinical implications related to nearly all major organs. It is a serious condition and the capacity for resorcinol to induce or contribute to existing (subclinical) hypothyroidism raises significant concern. Alterations of foetal development, in particular brain development as a consequence of low maternal levels in TH, have been associated with serious adverse health outcomes.

6.3.2.3 Irreversibility of the effects and delay of health effects

The effects of resorcinol on the induction of hypothyroidism and goitre in humans are reversible as demonstrated by reversal of effects in several human cases after cessation of exposure to resorcinol. In experimental animals, an effect on thyroid weight is observed in females at the end of the exposure period in the 90-day gavage study (Unpublished study report, 2014a) and an opposite effect is observed after a four-week period without treatment.

Because of compensatory mechanisms, a sufficiently high and long exposure is needed before the adverse effects related to disruption of synthesis of thyroid hormones are induced. In human cases, a delay of 3 months to 13 years is observed before the induction of severe hypothyroidism and its highly specific symptom, goitre. These outcomes are considered as an extreme manifestation of TH dysregulation and more moderate hypothyroidism is expected to be induced by lower durations and doses. Although serious, moderate hypothyroidism is however most likely more difficult to detect because symptoms are generally nonspecific and a delay in the detection of adverse effects is also expected. In the absence of biochemical investigations, latent or moderate forms may be underdiagnosed until more severe and specific symptoms appear. A delay is predicted in

any case in the onset of the adverse effect because of compensatory capacities that can counteract the effect up to a certain point.

The delay in the manifestation of adverse effects is also illustrated by human cases of mild TPO deficiency (about 30% residual activity) caused by specific mutations of the TPO gene (Narumi *et al.*, 2017). Although not detected at birth based on TSH-based screening, a goitre is detected between the ages of 8 to 19 years indicating development of hypothyroidism in the long-term.

Altogether, although generally considered reversible, hypothyroidism raises concern because of the delay in the onset of symptoms. Effects can be compensated in the short term but it may result in severe forms that appear after long-term exposure. In addition, less severe forms are expected to be more difficult to identify because symptoms are generally unspecific. This results in delays in diagnosis and treatment.

In contrast, the neurodevelopmental effects expected as a consequence of maternal low concentration of T4 have consequences that are observed later in life, without a direct exposure. They are considered as permanent and irreversible and raise an additional concern.

Due to the long time required for the effect of decreased TH synthesis to be expressed and/or the long term delayed effect link to alterations of thyroid dependent developmental processes, risk management measures and prevention of exposure may not be taken in time. Moreover, the latency and irreversibility of the effects may also pose some concern due to the fact that they may also affect future generations. **The difficulty posed by latency both in terms of detection of the effect and induction of developmental effects is considered as an additional concern.**

6.3.2.4 Quality of life affected

Hypothyroidism encompasses a wide range of manifestations as described above and they can significantly impact the quality of life. One of the most common symptoms is fatigue and loss of energy. Chronic fatigue syndrome has been strongly associated with low quality of life (Pedersen, 2019). Fatigue may have an impact on the ability to cope with daily burdens, on involvement in social activities as well as efficiency at work. Other symptoms such as muscle cramps, or cold sensitivity can impact well-being. Weight gain, dry skin and hair loss can impact the physical appearance and self-esteem if negatively perceived. Weight gain may also be a risk factor for other health problems. Other symptoms include poor memory, slowed thinking, anxiety, and depression and can have an impact on daily life by impairing the ability of an individual to effectively and autonomously cope with daily tasks and with difficulties.

Neurobehavioural effects as a consequence of maternal depletion in thyroid hormones can also lead to poorer cognitive functions. It may occur through many various forms such as weak memory, disrupted learning capacities, etc. In children and adolescents it may also affect school performance and may contribute to situations of school failure that may impact children's self-esteem as well as compromise future educational and occupational achievement (Trasande *et al*., 2016). It may also create a significant source of worries in parents. At a population level, this effect could lead to a global displacement of the Gaussian IQ distribution impacting the average IQ of the population.

The adverse effects induced or enhanced by resorcinol may therefore lead to a reduced quality of life.

6.3.2.5 Societal concern

The incidence of pathologies of the thyroid and of conditions in relation to thyroid dysfunction have been observed to increase in recent years.

The annual number of thyroid hormone prescription items dispensed in England more than doubled from 1998 to 2007 (Mitchell *et al.,* 2009), and in the Tayside region in Scotland, the prevalence of treated hypothyroidism increased by 63% from 1994 to 2001 (Leese *et al.,* 2008). In Denmark, the incidence rate of treated hypothyroidism increased by 80% from 1997 to 2008 (Cerqueira *et al*., 2011). Asvold *et al.* (2013) examined changes in prevalence of hypothyroidism in a Norwegian county from 1995-1997 to 2006-2008. Although the overall (treated and untreated) prevalence of hypothyroidism was stable, the prevalence of treated hypothyroidism increased by 60% from 5.0 to 8.0% among women and doubled from 1.0 to 2.0% in men. More extensive detection and treatment of subclinical hypothyroidism partly explain these changes but environmental factors may also contribute.

Since the early 1970s, the incidence of thyroid cancer has also more than doubled in most industrialised countries. European countries reported increases in incidence between 5.3% (Switzerland) and 155.6% (France). The increased incidence has in particular been observed in females, children and young adults. In fact, thyroid cancer has become the fastest rising type of cancer among women in North America within the last two decades (Kortenkamp *et al*., 2012). Overall, thyroid cancer has an incidence rate in men of 2.9/100 000 and 7/100 000 in women in Europe (Kortenkamp *et al*., 2012). In 2011, the incidence rates in France were 4.4 in men and 13.65/100 000 in women (http://globocan.iarc.fr/).

Several studies have shown that after decades of increase, IQ scores have tended to stabilise or decline since the end of the 1990s. A decrease was observed in Danish male conscripts between 1998 and 2003/2004 (Teasdale & Owen, 2005 and 2008), and in 14-year old teenagers in the United Kingdom (Flynn 2009). The causes of these decreases are difficult to interpret because IQ scores are influenced by many socioeconomic and cultural factors i.e. nutritional, educational environments. Considering the association of maternal thyroid function during early pregnancy with offspring IQ (Korevaar et al., 2016; US EPA, 2019), a contribution of thyroid-disrupting substances is possible.

An increase in the prevalence of neurodevelopmental disorders such as autism and attention deficit hyperactivity disorder (ADHD) has been observed over the past decades. Increased awareness and a shift in diagnosis may have contributed to the higher prevalences. However, they do not offer a sufficient explanation for these findings. A number of genetic and environmental factors are associated with these pathologies. Among them, there is currently considerable concern about a potential relationship between the increasing prevalence of neurodevelopmental disorders and effects on the homeostasis and action of TH (Jugan *et al*., 2010; de Cock *et al.*, 2012, Fini & Demeneix 2019). This relationship was discussed for the first time by Colborn (2004), who highlighted the difficulty of establishing this causal link, and the need to develop improved tools to detect and assess thyroid-disrupting chemicals (TDCs).

A number of genetic and environmental factors are also known to be involved in neuro-degenerative diseases such as multiple sclerosis, Alzheimers and Parkinsons. Among them, thyroid disruption via crosstalk with other signalling systems has been suggested as a possible contributory factor for their increased incidences (Fini & Demeneix, 2019).

Overall, thyroid-disrupting chemicals may have a role in the increased incidence of pathologies of the thyroid or conditions associated with thyroid dysfunction, in particular hypothyroidism, thyroid cancer and neurodevelopmental disorders. This raises a societal concern.

In its state-of-the-art-report Kortenkamp *et al*. (2012) reports that although population-based statistics are not generally available for neurodevelopmental outcomes, surveys indicate that, for example in the US, several hundred thousand children have disabling childhood mental health conditions including mental retardation, learning disabilities, autism and attention deficit hyperactivity disorder (ADHD). Endocrine disruption is unlikely to be the only cause of such trends but may significantly contribute. In its assessment of health costs associated with ED chemicals, Rijk *et al*. (2016) stated that neurodevelopmental and behavioural diseases and disorders include several pervasive disorders that persist for a lifetime, thereby leading to prolonged costs. They concluded that these disorders comprise the largest contributors to the total EDC-associated socio-economic cost estimates. Especially the contribution of IQ loss dominates the cost, accounting for between 32 and 184 billion € per year for the EU28 (indirect cost). HEAL (2014) also provided an evaluation of neurological disorders affecting child brain development and behaviour due to ED chemicals but only focusing on autism and ADHD. The study reported a direct cost of between 4.5 and 11.3 billion € per year for the EU28, autism being the major contributor. Bellanger *et al*. (2015) estimated a cost of €80-400 million for ED-related autism spectrum disorders.

The possible contribution of resorcinol to this burden has not been calculated but cannot be excluded.

The significant costs for healthcare systems and for society at large when also considering possible indirect costs of future decreased productivity, is a major societal concern.

6.3.2.6 Is derivation of a 'safe concentration' possible?

The ED MoA of resorcinol through inhibition of TPO appears as a simple mode of action for which characterisation of the dose-response and determination of safe levels should be possible. However**, a number of uncertainties have emerged considering the resorcinol data as detailed below as well as considering recent developments in the understanding of the consequences of thyroid disruption.**

Uncertainties

- Uncertainties regarding factors influencing potency of TPO inhibition

Although comparison with known TPO inhibitors such as MMI and PTU allows to conclude that resorcinol is a potent TPO inhibitor *in vitro*, the variability in study results raises uncertainties on the factors that can influence the effect of resorcinol on TPO.

Cooksey *et al*. (1985) also reported *in vitro* that TPO inhibition is not dose-dependent with stronger effects at lower doses. An influence of pH and of iodine concentration in the media is observed in a study performed on LPO (Divi & Doerge 1994). At low concentration, iodine slightly enhanced inhibition by resorcinol while high concentrations reduced the inhibitory effect of resorcinol on TPO activity. This is consistent with the effects observed in Cooksey *et al*. (1985) in rats fed with a low-iodine diet. It has to be noted that recent epidemiological studies raise concern on iodine levels in the human population. In the study by Steinmaus *et al*. (2016) performed in South California, a large proportion of the women (74%) had urinary iodine levels below the recommended median level (150 µg/L) for pregnancy. Rayman & Bath (2015) reports that the United Kingdom is now classified as mildly iodine deficient by the World Health Organization, based on a 2011 national study of 14-15-year-old schoolgirls. In addition, it is well established that pregnancy, a critical stage for thyroid-dependant neurodevelopment, is often associated with insufficient iodine intake to cover pregnancy requirements (Glinoer *et al*., 1990).

Iodine might be an important modulator of resorcinol action as iodine concentration in the thyroid follicle may be variable depending on iodine dietary intake and may also be modulated through increased TSH and the subsequent increased expression of NIS in response to thyroid disruption. Therefore, **the potency of TPO inhibition can be modulated by several factors, in particular iodine.**

- Limited characterisation of the consequences linked to TH disruption

Considering the wide range of functions influenced by TH, it is also highly challenging to fully characterise these effects and their dose-response in experimental studies. In particular, neurodevelopmental effects are generally considered as the most sensitive. There is some evidence indicating a possible neurodevelopmental effect of resorcinol but that has not been investigated in detail.
These elements therefore raise concern on the possibility to establish safe levels for resorcinol in relation to its thyroid-disrupting effects.

- Uncertainty in dose-response based on human data

A limited number of human cases have been reported and correspond to severe hypothyroidism in response to conditions of high systemic exposure. Symptoms of hypothyroidism may be diffuse and non-specific so that less severe conditions may have been under-detected and thus under-reported. No epidemiological studies have been conducted to systematically investigate the occurrence of thyroid pathologies in relation to the use of resorcinol as an ointment and the incidence of less severe manifestations is not known. Establishing a safe level based on human data is highly uncertain.

Lack of information on internal levels of exposure (plasma concentration) in human cases and incidence of less severe hypothyroidism precludes the estimation of a dose-response relationship in humans.

- Uncertainties regarding toxicokinetic considerations

Experimental toxicokinetic data demonstrates that resorcinol is efficiently absorbed within 24 h by gavage in corn oil (see Kim & Matthews, 1987 in section 4.3) and efficiently metabolised both by gavage and the dermal route within 24 h. Studies by the oral route when administered in diet or drinking water provide a consistent picture in the pattern of effects detected and more or less pronounced. For the negative response observed in the gavage studies conducted by NTP (1992), a role of the vehicle or a contribution of sublingual absorption that bypasses direct first-pass hepatic metabolisation is possible. The difference between bolus administration by gavage versus more continuous exposure by diet, drinking water, inhalation and (sub)cutaneous routes may also explain the differences but there is no clear understanding on how it impacts systemic exposure.

In human case reports, doses of resorcinol are estimated to be 2 to 140 mg/kg/d. Dermal absorption of unconjugated resorcinol is considered high due to application on damaged skin. However, even presuming 100% absorption, these doses are below the maximal doses (225 mg/kg/d) used in the chronic rat and mouse studies performed by gavage by NTP. No effect on thyroid was observed in the NTP study whereas absorption by gavage is also efficient. Despite the very high absorption rate, a gavage route with a potentially high first pass hepatic effect can lead to low bioavailability whereas a route that partly bypasses the hepatic first pass effect can lead to higher bioavailability. However, available data do not allow to confirm or dismiss these possible explanations.

The discrepancy between experimental results depending on the route of administration and/or vehicle is not fully understood. In particular, internal exposure and toxicokinetic differences depending on route of exposure and vehicle have not been fully characterised. Comparing the severity of effects

between rodents and humans, a higher sensitivity of humans or certain humans cannot be excluded.

Due to efficient metabolisation, systemic levels of resorcinol at steady state are expected to be low. This is confirmed in the two experimental studies that measured resorcinol levels in blood:

- In the two-generation study via drinking water (Unpublished study report 2005a): in F1 animals after 143 to 155 days of dosing, levels of free resorcinol were detected in the blood of only a few animals within 1h after light onset (1/10 M and 2/10 F) at the highest dose of 3000 mg/L in drinking water (LOQ=0.1 µg/mL, one single time of blood collection).

  In F1, indications of increased TSH (+18%, not significant) in males and of decreased T4 (-19%, not significant) in females were observed in the high dose group of 3000 mg/L.

- In the 90-day gavage study ((Unpublished study report 2004a), plasma levels were quantifiable only at 0.5 to 2 hours for the 80 and 250 mg/kg/d groups at week 13 of exposure (LOQ=0.5 µg/mL). AUC in females was approximately 3 times higher than in males.

  Decreased thyroid weight was observed in high dose females.

Depending on the route of administration, effects can therefore be induced by low systemic concentrations of resorcinol in experimental studies.

It is also noted that although dermal absorption of resorcinol was previously considered low (approximately 2%), recent *in vitro* studies (Genies *et al.,* 2019a, 2019b; Hewitt *et al*., 2020) have demonstrated a much higher transcutaneous penetration in PBS (50-70%) and raise important uncertainties regarding this parameter. The role of hydrophilic versus lipophilic vehicle has also not been elucidated. Absorption by inhalation, another relevant route of exposure for humans has not been investigated.

Large uncertainties remain on the level of systemic exposure to resorcinol that induces effects on the thyroid and on the level of systemic exposure to resorcinol after different routes of exposure.

<u>Recent understanding of the consequences of small changes in maternal TH levels</u>

The importance of even modest changes in TH during pregnancy has emerged and strengthened recently, in particular from studies on pregnant women whose children have been subsequently assessed with standardised intelligence tests (Gilbert *et al*., 2012).

In 2011, the American Thyroid Association defined maternal hypothyroxinemia as low serum free thyroxine (FT4) levels (<5th or <10th percentile) existing in conjunction with normal serum free triiodothyronine (FT3) or thyroid stimulating hormone (TSH) levels during pregnancy (Stagnaro-Green *et al*., 2011).

A recent review by Min *et al*., 2016 concludes that unequivocal evidence demonstrates that maternal hypothyroxinemia leads to negative effects on foetal brain development, increasing the risks for cognitive deficits and poor psychomotor development in resulting progeny.

A similar review by Henrichs *et al.* (2013) concluded that clinical and epidemiological studies suggest that maternal hypothyroxinemia in the first half of pregnancy but not later

in pregnancy impairs cognitive development in infancy and childhood. Sharlin *et al*. (2010) also criticised the general concept that the developing brain possesses potent compensatory mechanisms to protect it from small (or even moderate) changes in circulating levels of TH. For example, delayed neurobehavioural development is observed in 18-month old children from mothers that were mildly hypothyroxinemic during the first 12–14 gestational weeks (Berbel *et al*., 2009). Children of women with hypothyroxinaemia (FT4 levels below the 10th percentile with TSH levels within the reference range) during the first trimester of pregnancy are at risk of having a delay in both mental and motor development at the age of 1 year, as well as at 2 years (Pop *et al*., 2003).

In support of this, rodent models provide direct evidence of neurodevelopmental damage induced by maternal hypothyroxinemia, including dendritic and axonal growth limitation, neural abnormal location, and synaptic function alteration. In the offspring of rats with experimentally induced transient and moderate hypothyroxinemia during early prenatal development, maternal free T4 deficiency had an irreversible impact on neurogenesis (Auso *et al*., 2004; Lavado-Autric *et al.,* 2003). During this prenatal period, neurogenesis takes place and radial neurons migrate into the developing (neo)cortex and the hippocampus. Finally, gliogenesis has been shown to be tightly related to serum T4 levels during development, even at very small reductions in serum T4 (Sharlin *et al.* 2010). The complex interconnectedness of brain regions required for normal function could be impaired by effects on all of these processes simultaneously or in sequence, and across multiple brain regions. Maternal thyroid deficiencies during these processes further affect visual processing, development of fine motor skills, IQ, and selective learning problems (Zoeller & Rovet, 2004; Zoeller *et al.,* 2007, US EPA, 2019). TH action was reduced despite activation of 'compensatory' and 'adaptive' mechanisms (increase in serum TSH, in type 2 deiodinase mRNA expression and enzyme activity in the brain, and in the expression of the mRNA encoding the T3 transporter MCT8). Taken together, these findings contradict the concept that adaptive mechanisms in the developing brain can compensate for low circulating levels of TH during development. Morreale de Escobar *et al.* (2000) extrapolated these findings to humans and argue that the first half of pregnancy constitutes a sensitive period in which mild free T4 insufficiency may particularly affect neuronal and structural development in the foetal brain. Remarkably, the human evidence as reviewed by Henrichs *et al.* (2013) supports the timing of this T4-specific sensitive period of the developing foetal brain.

Gilbert *et al.* (2012) suggested that the timing, duration and severity (Figure 7 below) of the TH insufficiency determine the type of deficit produced.

Figure 7: Gradient of thyroid hormone insufficiency.
Both human and animal studies indicate that moderate degrees of thyroid hormone insufficiency can impact brain structure and function and suggest that this may be true as well across the range of subclinical hypothyroidism. Source: Gilbert *et al.* 2012. The figure was deleted due to potential copyright reasons.

In the context of the assessment of perchlorate, US EPA (2019) recently performed a comprehensive review of epidemiological studies that evaluated maternal thyroid hormone levels in early pregnancy and neurodevelopmental outcomes (these are not studies evaluating perchlorate exposure). The US EPA concluded as follows:

> *"Evaluating these results as a whole demonstrates that in different populations, at different ages for neurodevelopmental assessment, and at various cut points for fT4, there is a significant difference in performance on global cognitive tests when comparing the offspring of hypothyroxinemic women to those of non-hypothyroxinemic women (Costeira et al., 2011; Ghassabian et al., 2014; Júlvez et al., 2013; Korevaar et al., 2016; Li et al., 2010; Pop et al., 1999, 2003). These findings are supported by several systematic reviews and meta-analyses*

including Fan and Wu (2016), Wang et al. (2016), and Thompson et al. (2018). Fan and Wu (2016) and Wang et al. (2016) found that hypothyroxinemia was associated with a 5.7-point lower score on intelligence tests and a three-fold increased risk of delayed cognitive development in children, respectively. Thompson et al. (2018) found that maternal hypothyroxinemia is associated with increased risk of cognitive delay, intellectual impairment, or lower scores on performance tests, but they did not find this association with ADHD or autism.

The only studies that did not find a statistically significant effect on any of the evaluated endpoints in Table 31 are Grau et al. (2015) and Hales et al. (2018). As previously discussed Grau et al. (2015) has a high hypothyroxinemic cut point (13.7 pmol/L), and individuals are not iodine deficient. Hales et al. (2018) hypothesizes that the lack of congruence in their findings may be related to varying definitions of suboptimal thyroid function, lack of universal pregnancy-specific reference ranges for thyroid function tests, and the application of various tools to measure cognition in children across the age spectrum.

Additionally, studies identified in the literature review […] also associated maternal hypothyroxinemia with an offspring's increased risk of schizophrenia (Gyllenberg et al., 2016), ADHD (Modesto et al., 2015), expressive language delay (Henrichs et al., 2010), and other outcomes (Finken et al., 2013; Kooistra et al., 2006; Noten et al., 2015; Päkkilä et al., 2015; Román et al., 2013; van Mil et al., 2012; Oostenbroek et al., 2017). These studies demonstrate the sensitivity of the offspring of hypothyroxinemic mothers to adverse neurodevelopmental effects.

[…]

Overall, the results of this literature review lend support to the concept that maternal FT4, especially in the hypothyroxinemic range, is critical to proper offspring's neurodevelopment. Across different age ranges and neurodevelopmental indices, the impact of altered FT4 is seen even with small incremental changes in FT4 *(and in populations with FT4 across the "normal" range)."*

Sensitive populations to thyroid-disruption

- *Pre-existing thyroid disorder*

Hypothyroidism is among the most common of endocrine conditions and resorcinol can aggravate existing conditions.

The prevalence of overt hypothyroidism in the general population varies between 0.2% and 5.3% in Europe, depending on the definition used (Chaker *et al*., 2017). The most common cause of hypothyroidism is chronic autoimmune thyroiditis.

Subclinical hypothyroidism is more common in women than in men and in older people than in younger ones. The prevalence of latent/subclinical hypothyroidism is between 3% and 10%, according to epidemiological studies that have been carried out in the USA, the United Kingdom, and Denmark. As persons with latent hypothyroidism are often asymptomatic, the diagnosis is often made incidentally in routine laboratory testing. (Schuebel *et al*., 2017). Epidemiological surveys and/or meta-analysis studies estimate a high incidence and/or prevalence of undiagnosed thyroid function abnormalities in Europe (Garmendia Madariaga *et al*., 2014) including in pregnant women (Andersen, 2019). According to the meta-analysis of Garmendia Madariaga *et al*. (2014), nearly 11% of Europeans have thyroid dysfunction, and of special concern, only about half of them are aware of their condition. Most of these patients (approximately 4.5 of 5) have mild thyroid disease, with almost two-thirds of them having subclinical hypothyroidism, the leading

cause of thyroid dysfunction. Given the mildness of the clinical manifestations of subclinical hypothyroidism, the diagnosis is often overlooked.

Any additional thyroid disturbance by resorcinol may in some instances counteract part of the compensatory mechanisms developed by the thyroid gland, namely increased expression of TPO and could aggravate an existing condition that is of relatively common prevalence although often undiagnosed.

- *High maternal sensitivity to disruption of TH levels during pregnancy*

Pregnancy is a period of specific sensitivity to disruption of TH. It is considered that physiologic changes associated with pregnancy require an increased availability of thyroid hormones by 40% to 100% to meet the needs of the mother and the foetus during pregnancy. Pregnancy has an effect on thyroid functions with significant changes in iodine metabolism and clearance, and serum thyroid binding proteins (Soldin, 2006). Thyroid-related pathological changes aggravated by pregnancy, and some obstetric conditions, such as gestational trophoblastic disease or hyperemesis gravidarum, may impact maternal-foetal thyroid hormone balance.

Hypothyroxinemia can be induced in response to several factors, such as mild iodine deficiency and certain thyroid diseases. A contributive role of environmental endocrine disrupters has been also hypothesised (Min *et al.,* 2016). Compared to clinical or subclinical hypothyroidism, hypothyroxinemia is more commonly found in pregnant women and can be unveiled by pregnancy. Depending on the different cut-offs used to define hypothyroxinemia, studies from around the world and from populations with various iodine levels, reports range from 1.3 to 23.9% of maternal hypothyroxinemia. In iodine-sufficient populations, the prevalence of maternal hypothyroxinemia is considered to be 1-2% (Dosiou & Medici, 2017).

Pregnancy is therefore a period that is highly sensitive to disruption of TH regulation because of a higher need for TH and physiological changes affecting their toxicokinetics. Pregnancy is likely to be a period of sensitivity to the alteration of TH regulation by resorcinol, with consequences for the neurodevelopment of the offspring that can be affected even by small changes.

A number of vulnerable populations may therefore be of particular concern. Sensitive populations include those with undiagnosed subclinical hypothyroidism, marginal dietary iodine deficiency, pregnant women, the developing foetus, the newborn, and young infants - all of which may be particularly susceptible to TH disruption induced by resorcinol. Establishing safe levels for these particularly sensitive populations is surrounded with large uncertainties due to the lack of agreed parameters to identify such persons and high symptom variability in relation to the physiological status.

6.3.2.7 Overall assessment of the equivalent level of concern

- Human cases have been identified under specific conditions and this may raise some questions on their relevance for actual conditions of human exposure. The effects induced by resorcinol in humans and in experimental animals are complementary in the demonstration that they cannot be considered as specific to exceptional conditions of exposure, in particular because some populations and periods of exposure can be associated with specific sensitivity. Besides, the

possibility that resorcinol may induce effects in humans under common conditions of exposure can not be excluded on a toxico-kinetic basis.

- Hypothyroidism has clinical implications related to nearly all major organs. It is a serious condition and the capacity for resorcinol to induce or contribute to existing (subclinical) hypothyroidism raises significant concern. Alterations of foetal development, in particular brain development as a consequence of low maternal levels in TH, have been associated with serious adverse health outcomes.

- Although generally considered reversible, hypothyroidism raises concern because of the delay in the onset of symptoms. Effects can be compensated for in the short-term but it may result in severe forms that appear after long-term exposure. In addition, less severe forms are expected to be more difficult to identify because symptoms are generally unspecific. This results in delays in diagnosis and treatment. In contrast, the neurodevelopmental effects expected as a consequence of maternal low concentration of T4 have consequences that are observed later in life, without a direct exposure. They are considered as permanent and irreversible and raise an additional concern. The difficulty posed by latency both in terms of detection of the effect and induction of developmental effects is considered as an additional concern.

- The adverse effects induced or enhanced by resorcinol may lead to a reduced quality of life.

- Thyroid-disrupting chemicals may have a role in the increased incidence of pathologies of the thyroid or conditions associated with thyroid dysfunction, in particular hypothyroidism, thyroid cancer and neurodevelopmental disorders. This raises a societal concern.

- A number of uncertainties to derive a 'safe concentration' have emerged considering the resorcinol data as detailed below as well as considering recent developments in the understanding of the consequences of thyroid disruption.

    - The potency of TPO inhibition can be modulated by several factors, in particular iodine levels.

    - Considering the wide range of functions influenced by TH (thyroid hormone), it is also highly challenging to fully characterise these effects and their dose-response in experimental studies. In particular, neurodevelopmental effects are generally considered as the most sensitive. There is some evidence indicating a possible neurodevelopmental effect of resorcinol but that has not been investigated in detail.

    - Lack of information on internal levels of exposure (plasma concentration) in human cases and on incidence of less severe hypothyroidism preclu de the estimation of a dose-response relationship in humans.

    - The discrepancy between experimental results depending on the route of administration is not fully understood. In particular, internal exposure and toxicokinetic differences depending on route of exposure and vehicle have not been fully characterised. Comparing the severity of effects between rodents and humans, a higher sensitivity of humans or certain humans cannot be excluded. Depending on the route of administration, some effects have been observed in experimental studies at doses associated with low systemic concentrations of resorcinol in experimental studies. Large uncertainties remain on the level of systemic exposure to resorcinol that induces effects on the thyroid function. Additional uncertainty remains on the level of systemic exposure to resorcinol after different routes of exposure.

- Hypothyroidism is among the most common of endocrine conditions and resorcinol can aggravate existing conditions. Any additional thyroid disturbance by resorcinol may in some instances counteract part of the compensatory mechanisms developed by the thyroid gland, namely increased expression of TPO, and could aggravate an existing condition that is of relatively common prevalence although often undiagnosed. Pregnancy is also a period that is highly sensitive to disruption of TH regulation because of a higher need for TH and physiological changes affecting their toxicokinetics. Besides, the importance of even modest changes in TH during pregnancy has emerged and strengthened recently. The results of a comprehensive review by the US EPA (2019) lend support to the concept that maternal FT4 (free T4), especially in the hypothyroxinemic range, is critical to proper neurodevelopment of the offspring. Across different age ranges and neurodevelopmental indices, the impact of altered FT4 is seen even with small incremental changes in FT4. Pregnancy is therefore likely to be a period of sensitivity to the alteration of TH regulation by resorcinol, with consequences for the neurodevelopment of the offspring that can be affected even by small changes. A number of vulnerable populations may therefore be of particular concern. Sensitive populations include those with undiagnosed subclinical hypothyroidism, marginal dietary iodine deficiency, pregnant women, the developing foetus, the newborn, and young infant – all of which may be particularly susceptible to TH disruption induced by resorcinol. Establishing safe levels for these particularly sensitive populations is surrounded with large uncertainties due to the lack of agreed parameters to identify such persons and high symptom variability in relation to the physiological status.

Some of the effects that resorcinol may induce in relation to its thyroid-disrupting potential are serious and are irreversible or may not be detectable without delay. They can impact on the quality of life and raise societal concern of a high and increasing burden. Most importantly, the difficulty to establish a safe level with sufficient certainty raises concern on the capacity to manage safe use of the substance in particular for sensitive populations and with the emergence of the understanding that small changes in maternal T4 can affect brain development of the offspring. Altogether, this gives rise to an equivalent level of concern to those of other substances listed in points (a) to (e) of Article 57 REACH**.**

Table 31: Summary of factors to be considered in ELoC assessment for the different ED-related effects identified

	Possible serious health effects?	Irreversibility and delay	Impact on quality of life	Societal concern	Difficulty to derive a safe concentration
Hypothyroidism	**YES** ▪ Wide range of physiological functions impacted	**NO** ▪ reversal of effects in human cases when exposure stop **YES** ▪ Delay in onset and detection of effects	**YES** ▪ Symptoms associated with poor quality of life	**YES** ▪ Increased incidence of pathologies related to thyroid homeostasis.	**YES** ▪ Associated with large uncertainties
Neuro-developmental effects of	**YES** ▪ Serious dysfunction	**YES** ▪ Effects induced via	**YES** ▪ Serious dysfunction	**YES** ▪ Increased incidence of	**YES** ▪ Recent review

maternal hypothyroxinemia	having consequences on the cognitive performance	*in utero* exposure with consequences later in children life → irreversible and appearing with a delay	having consequences on quality of life	neuro-developmental outcomes that represent a high burden for society	concluded that maternal TH insufficiency induces a gradient of neurobehavioural deficits

6.3.3 Conclusion on the hazard properties and equivalent level of concern assessment

ED properties of resorcinol relevant for human health

Resorcinol fulfils the definition of an endocrine disruptor relevant for human health as summarised in section 4.8 above on the following basis:

It is well established based on a series of case reports, where high doses of resorcinol were given, that severe hypothyroidism was diagnosed and reversed when exposure to resorcinol was stopped. This leads to the conclusion that exposure to resorcinol can affect the regulation of the thyroid function inducing hypothyroidism in humans. The endocrine disrupting properties of resorcinol have therefore been demonstrated in humans under these specific conditions of exposure.

The medical cases reporting these effects are patients that applied resorcinol to damaged skin for 9 out of 10 cases. However, considering that the skin was reported as intact in one additional case and that a skin absorption has been demonstrated *in vitro*, these data are considered relevant in the assessment.

In addition, findings consistent with the MoA (mode of action) of thyroid disruption via TPO (thyroperoxidase) inhibition are also reported in several experimental studies via drinking water. Similar findings reported in studies conducted by subcutaneous, dietary and inhalation routes provide supportive evidence. In particular histopathological changes in the thyroid and changes in the circulating levels of T3 (triiodothyronine) or T4 (thyroxine), are considered as adverse effects.

Inhibition of TPO by resorcinol, a key enzyme in the synthesis of thyroid hormones, is established *in vitro* in several experimental designs.

Altogether, the effects observed in humans and in some experimental studies are fully consistent with the MoA via TPO inhibition. Based on current knowledge, the biological plausibility of a causal link between inhibition of TPO, disruption of thyroid hormone levels and adverse effects linked to low thyroid hormones levels is strong. A recently validated AOP (adverse outcome pathway) described the relationship between inhibition of TPO, decreased T4 and neurodevelopmental alteration due to maternal low T4 concentration as having a high level of evidence for humans (AOP n°42). With the validation of this AOP by the OECD (Crofton *et al.*, 2019), further experimental data investigating (neuro)development are not necessary to confirm the effects observed in the preliminary two-generation study available in the registration dossier.

Based on a weight of evidence analysis of the available data, there is scientific evidence that resorcinol can have adverse effects on human health through thyroid disruption and fulfils the definition of an endocrine disruptor.

Overall assessment of an Equivalent level of concern

Due to the ED properties of resorcinol, it should be regarded as a substance of equivalent level of concern as specified in article 57 of REACH as summarised at the end of section 6.3.2.7 on the following basis:

- Human cases have been identified under specific conditions and this may raise some questions on their relevance for actual conditions of human exposure. The effects induced by resorcinol in humans and in experimental animals are complementary in the demonstration that they cannot be considered as specific to exceptional conditions of exposure, in particular because some populations and periods of exposure can be associated with specific sensitivity. Besides, the possibility that resorcinol may induce effects in humans under common conditions of exposure can not be excluded on a toxico-kinetic basis.

- Hypothyroidism has clinical implications related to nearly all major organs. It is a serious condition and the capacity for resorcinol to induce or contribute to existing (subclinical) hypothyroidism raises significant concern. Alterations of foetal development, in particular brain development as a consequence of low maternal levels in TH, have been associated with serious adverse health outcomes.

- Although generally considered reversible, hypothyroidism raises concern because of the delay in the onset of symptoms. Effects can be compensated for in the short-term but it may result in severe forms that appear after long-term exposure. In addition, less severe forms are expected to be more difficult to identify because symptoms are generally unspecific. This results in delays in diagnosis and treatment. In contrast, the neurodevelopmental effects expected as a consequence of maternal low concentration in T4 have consequences that are observed later in life, without a direct exposure. They are considered as permanent and irreversible and raise an additional concern. The difficulty posed by latency both in terms of detection of the effect and induction of developmental effects is considered as an additional concern.

- The adverse effects induced or enhanced by resorcinol may lead to a reduced quality of life.

- Thyroid-disrupting chemicals may have a role in the increased incidence of pathologies of the thyroid or conditions associated with thyroid dysfunction, in particular hypothyroidism, thyroid cancer and neurodevelopmental disorders. This raises a societal concern.

- A number of uncertainties to derive a 'safe concentration' have emerged considering the resorcinol data as detailed below as well as considering recent developments in the understanding of the consequences of thyroid disruption.

    - The potency of TPO inhibition can be modulated by several factors, in particular iodine levels.

    - Considering the wide range of functions influenced by TH (thyroid hormone), it is also highly challenging to fully characterise these effects and their dose-response in experimental studies. In particular, neurodevelopmental effects

are generally considered as the most sensitive. There is some evidence indicating a possible neurodevelopmental effect of resorcinol but that has not been investigated in detail.

- Lack of information on internal levels of exposure (plasma concentration) in human cases and on incidence of less severe hypothyroidism preclude the estimation of a dose-response relationship in humans.

- The discrepancy between experimental results depending on the route of administration is not fully understood. In particular, internal exposure and toxicokinetic differences depending on route of exposure and vehicle have not been fully characterised. Comparing the severity of effects between rodents and humans, a higher sensitivity of humans or certain humans cannot be excluded. Depending on the route of administration, some effects have been observed in experimental studies at doses associated with low systemic concentrations of resorcinol in experimental studies. Large uncertainties remain on the level of systemic exposure to resorcinol that induces effects on the thyroid function. Additional uncertainty remains on the level of systemic exposure to resorcinol after different routes of exposure.

- Hypothyroidism is among the most common of endocrine conditions and resorcinol can aggravate existing conditions. Any additional thyroid disturbance by resorcinol may in some instances counteract part of the compensatory mechanisms developed by the thyroid gland, namely increased expression of TPO, and could aggravate an existing condition that is of relatively common prevalence although often undiagnosed. Pregnancy is also a period that is highly sensitive to disruption of TH regulation because of a higher need for TH and physiological changes affecting their toxicokinetics. Besides, the importance of even modest changes in TH during pregnancy has emerged and strengthened recently. The results of a comprehensive review by the US EPA (2019) lend support to the concept that maternal FT4 (free T4), especially in the hypothyroxinemic range, is critical to proper neurodevelopment of the offspring. Across different age ranges and neurodevelopmental indices, the impact of altered FT4 is seen even with small incremental changes in FT4. Pregnancy is therefore likely to be a period of sensitivity to the alteration of TH regulation by resorcinol, with consequences for the neurodevelopment of the offspring that can be affected even by small changes. A number of vulnerable populations may therefore be of particular concern. Sensitive populations include those with undiagnosed subclinical hypothyroidism, marginal dietary iodine deficiency, pregnant women, the developing foetus, the newborn, and young infant – all of which may be particularly susceptible to TH disruption induced by resorcinol. Establishing safe levels for these particularly sensitive populations is surrounded with large uncertainties due to the lack of agreed parameters to identify such persons and high symptom variability in relation to the physiological status.

Some of the effects that resorcinol may induce in relation to its thyroid-disrupting potential are serious and are irreversible or may not be detectable without delay. They can impact on the quality of life and raise societal concern of a high and increasing burden. Most importantly, the difficulty to establish a safe level with sufficient certainty raises concern on the capacity to manage safe use of the substance in particular for sensitive populations and with the emergence of the understanding that small changes in maternal T4 can affect brain development of the offspring. Altogether, this gives rise to an equivalent level of concern to those of other substances listed in points (a) to (e) of Article 57 REACH**.**

Conclusion

Based on these elements, there is scientific evidence of probable serious effects to human health of resorcinol in relation to its thyroid-disrupting potential, which gives rise to an equivalent level of concern to those of other substances listed in points (a) to (e) of Article 57 REACH.

REFERENCES

Ahlersova E, Ahlers I, Kassayova M, Smajda B. (1997). Circadian oscillations of serum thyroid hormones in the laboratory rat: The effect of photoperiods. Physiol Res 46:443-449

Alshehri B, D'Souza DG, Lee JY, Petratos S, Richardson SJ. (2015) The diversity of mechanisms influenced by transthyretin in neurobiology: development, disease and endocrine disruption. J Neuroendocrinol. 27(5):303-23. doi:10.1111/jne.12271. Review. PubMed PMID: 25737004.

Andersen SL (2019). Frequency and outcomes of maternal thyroid function abnormalities in early pregnancy. Scand J Clin Lab Invest. 79(1-2):99-107. doi:10.1080/00365513.2018.1555858.

Arnott DG, Doniach I. (1952). The effect of compounds allied to resorcinol upon the uptake of. The Biochemical journal 50:473-479.10.1042/bj0500473

Asvold B, Vatten L, & Bjøro T (2013). Changes in the prevalence of hypothyroidism: the HUNT Study in Norway, European Journal of Endocrinology, 169(5), 613-620. https://eje.bioscientifica.com/view/journals/eje/169/5/613.xml

Auso E, Lavado-Autric R, Cuevas E, Del Rey FE, Morreale De Escobar G, Berbel P. (2004). A moderate and transient deficiency of maternal thyroid function at the beginning of fetal neocorticogenesis alters neuronal migration. Endocrinology 145:4037-4047.10.1210/en.2004-0274

Bauer (1985) Personal communication [cited in WHO, 2006].

Bassett JH, Williams GR. (2016). Role of thyroid hormones in skeletal development and bone maintenance. Endocr Rev 37:135-187.10.1210/er.2015-1106

Beekhuijzen M, Rijk JCW, Meijer M, de Raaf MA, Pelgrom S. (2019). A critical evaluation of thyroid hormone measurements in OECD test guideline studies: Is there any added value? Reprod Toxicol 88:56-66.https://doi.org/10.1016/j.reprotox.2019.07.014

Bellanger M, Demeneix B, Grandjean P, Zoeller RT, Trasande L (2015). Neurobehavioral deficits, diseases, and associated costs of exposure to endocrine-disrupting chemicals in the European Union. The Journal of Clinical Endocrinology and Metabolism, 100(4), 1256-1266.

Berbel P, Mestre JL, Santamaria A, Palazon I, Franco A, Graells M, González-Torga A, de Escobar GM (2009). Delayed neurobehavioral development in children born to pregnant women with mild hypothyroxinemia during the first month of gestation: The importance of early iodine supplementation. Thyroid 19:511-519.10.1089/thy.2008.0341

Berthezene F, Fournier M, Bernier E, Mornex R. (1973). Resorcin induced hypothyroidism. Report of 2 cases. Lyon Med 230:319-323

Berthezene F, Perrot L, Munari Y, Ponsin G. (1979). Multiple effects of resorcinol on thyroid function. Ann Endocrinol (Paris) 40:67-68

Bianco AC, Anderson G, Forrest D, Galton VA, Gereben B, Kim BW, Kopp PA, Liao XH, Obregon MJ, Peeters RP, Refetoff S, Sharlin DS, Simonides WS, Weiss RE, Williams GR (2014). American Thyroid Association Task Force on Approaches and Strategies to Investigate Thyroid Hormone Economy and Action. American Thyroid Association Guide to investigating thyroid hormone economy and action in rodent and cell models. Thyroid ;24(1):88-168. doi: 10.1089/thy.2013.0109.

Biondi B. (2013). The normal TSH reference range: what has changed in the last decade? J Clin Endocrinol Metab. 98(9):3584-7. doi: 10.1210/jc.2013-2760

Boeck C (1915). Fall von tödlicher Resorzinvergiftung bei äusserlicher Anwendung des Mittels. Dermatol. Wschr. 60:449-453

Bontemps H, Mallaret M, Besson G, Bochaton H, Carpentier F (1995). Confusion after topical use of resorcinol. Arch Dermatol. 1995 Jan;131(1):112.

Brandt K. (1986). Final report on the safety assessment of 2-methylresorcinol and resorcinol. J Am Coll Toxicol 5:167-203

Bull G, Fraser R. (1950). Myxœdema from resorcinol ointment applied to leg ulcers. The Lancet 255:851-855.10.1016/S0140-6736(50)90689-1

CANTOX (2000) Resorcinol: toxicology review and risk assessment with emphasis on thyroidal effects. Bridgewater, NJ, CANTOX Health Sciences International, pp. 1–29. [Quoted in WHO, 2006]

Cerqueira C, Knudsen N, Ovesen L, Laurberg P, Perrild H, Rasmussen LB & Jorgensen T (2011). Doubling in the use of thyroid hormone replacement therapy in Denmark: association to iodization of salt? European Journal of Epidemiology 26 629–635. (doi:10.1007/s10654-011-9590-5)

Chaker L, Bianco AC, Jonklaas J, Peeters RP. (2017). Hypothyroidism. Lancet (London, England) 390:1550-1562.10.1016/S0140-6736(17)30703-1

Cheymol J, Gay Y, Lavedan JP. (1951). Antithyroid action of diphenols. C R Seances Soc Biol Fil 145:999-1001

Choksi NY, Jahnke GD, St Hilaire C, Shelby M. (2003). Role of thyroid hormones in human and laboratory animal reproductive health. Birth Defects Res B Dev Reprod Toxicol 68:479-491.10.1002/bdrb.10045

Colborn T (2004). Neurodevelopment and endocrine disruption. Environ Health Perspect ;112(9):944-9. Review.

Cooksey RC, Gaitan E, Lindsay RH, Hill JB, Kelly K. (1985). Humic substances, a possible source of environmental goitrogens. Organic Geochemistry 8:77-80.10.1016/0146-6380(85)90054-3

Cooper DS, Saxe VC, Meskell M, Maloof F, Ridgway EC (1982). Acute effects of propylthiouracil (PTU) on thyroidal iodide organification and peripheral iodothyronine deiodination: correlation with serum PTU levels measured by radioimmunoassay. J Clin Endocrinol Metab. 1982 54(1):101-7.

Cooper DS, Kieffer JD, Halpern R, Saxe V, Mover H, Maloof F, Ridgway EC (1983). Propylthiouracil (PTU) pharmacology in the rat. II. Effects of PTU on thyroid function. Endocrinology 113:921-928.

Costeira MJ, Oliveira, P., Santos, N. C., Ares, S., Sáenz-Rico, B., Morreale de Escobar, G., & Palha, J. A. (2011). Psychomotor development of children from an iodine-deficient region. The Journal of Pediatrics, 159(3), 447-453. doi:10.1016/j.jpeds.2011.02.034

Coval ML, Taurog A. (1967). Purification and iodinating activity of hog thyroid peroxidase. J Biol Chem 242:5510-5523

Crofton K, Gilbert M, Paul Friedman K, Demeneix B, Marty MS, Zoeller TR (2019). Adverse Outcome Pathway on inhibition of Thyroperoxidase and subsequent adverse

neurodevelopmental outcomes in mammals, OECD Series on Adverse Outcome Pathways, n° 13, Éditions OCDE, Paris, https://doi.org/10.1787/ea5aa069-en

Danish Ministery of Environment. (2004) Substance flow analysis of Resorcinol. Schmidt A and Poulsen J. Environmental Project Nr. 942 2004.

de Cock M, Maas YG, van de Bor M (2012). Does perinatal exposure to endocrine disruptors induce autism spectrum and attention deficit hyperactivity disorders? Review. Acta Paediatr ;101(8):811-8. doi:10.1111/j.1651-2227.2012.02693.x.

De Coster S, van Larebeke N. (2012). Endocrine-disrupting chemicals: Associated disorders and mechanisms of action. J Environ Public Health 2012:713696-713696.10.1155/2012/713696

Delzell E (2000) Clinical and epidemiologic research on resorcinol. Submitted to the Resorcinol Task Force [Appendix A in CANTOX, 2000].

DiNardo JC, Picciano JC, Schnetzinger RW, Morris WE, Wolf BA. (1985). Teratological assessment of five oxidative hair dyes in the rat. Toxicol Appl Pharmacol 78:163-166.10.1016/0041-008X(85)90316-3

Divi RL, Doerge DR. (1994). Mechanism-based inactivation of lactoperoxidase and thyroid peroxidase by resorcinol derivatives. Biochemistry 33:9668-9674.10.1021/bi00198a036

Doniach I, Fraser R. (1950). Effect of resorcinol on the thyroid uptake of i131in rats. The Lancet 255:855-856.10.1016/S0140-6736(50)90690-8

Doniach I, Logothetopoulos J. (1953). The goitrogenic action of resorcinol in rats. Br J Exp Pathol 34:146-151

Dosiou C, Medici M. (2017). Management of endocrine disease: Isolated maternal hypothyroxinemia during pregnancy: Knowns and unknowns. Eur J Endocrinol 176:R21-R38.10.1530/EJE-16-0354

Dressler WE (1999). Hair dye absorption. R.L. Bronaugh, H.I. Maibach (Eds.), Percutaneous Absorption: Drugs-Cosmetics-Mechanisms-Methodology, 3rd Edition, Marcel Dekker, New York (1999), pp. 685-716

Dumont JE, Lamy F, Roger P, Maenhaut C (1992). Physiological and pathological regulation of thyroid cell proliferation and differentiation by thyrotropin and other factors. Physiol Rev. 72(3):667-697. doi:10.1152/physrev.1992.72.3.667

Durham E, Howie RN, Parsons T, Bennfors G, Black L, Weinberg SM, Elsalanty M, Yu JC, Cray JJ Jr. (2017). Thyroxine exposure effects on the cranial base. Calcif Tissue Int 101:300-311.10.1007/s00223-017-0278-z

EC (2002). Study on the scientific evaluation of 12 substances in the context of endocrine disrupter priority list of actions. European Commission. Produced by WRc-NSF. Authors: I Johnson and P Harvey. Ref: UC 6052 https://ec.europa.eu/environment/chemicals/endocrine/pdf/wrc_report.pdf

ECHA (2011). Guidance on information requirements and chemical safety assessment Chapter R.4: Evaluation of available information Reference: ECHA-2011-G-13-EN Publ.date: December 2011 https://echa.europa.eu/documents/10162/13643/information_requirements_r4_en.pdf/d6395ad2-1596-4708-ba86-0136686d205e

ECHA (2012). Identification of substances as SVHCs due to equivalent level of concern to CMRs (Article 57(f)) – sensitisers as an example. Document discussed at 11th CARACAL meeting (Nov 2012)

ECHA (2016). Practical Guide: How to use alternatives to animal testing to fulfil your information requirements for REACH registration. Version 2.0 July 2016 https://echa.europa.eu/documents/10162/13655/practical_guide_how_to_use_alternatives_en.pdf/148b30c7-c186-463c-a898-522a888a4404

ECHA (2017). Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7c: Endpoint specific guidance. Version 3.0. June 2017. https://echa.europa.eu/documents/10162/13632/information_requirements_r7c_en.pdf/e2e23a98-adb2-4573-b450-cc0dfa7988e5

ECHA/EFSA (2018) Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009. European Chemical Agency (ECHA) and European Food Safety Authority (EFSA) with the technical support of the Joint Research Centre (JRC). Authors: Niklas Andersson Maria Arena Domenica Auteri Stefania Barmaz Elise Grignard Aude Kienzler Peter Lepper Alfonso Maria Lostia Sharon Munn Juan Manuel Parra Morte Francesca Pellizzato Jose Tarazona Andrea Terron Sander Van der Linden. EFSA Journal 8(1):1411 https://doi.org/10.2903/j.efsa.2018.5311 http://onlinelibrary.wiley.com/doi/10.2903/sp.efsa.2018.EN-1447/full

EFSA (2010). Scientific Opinion on the use of Resorcinol as a food additive. http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2010.1411/epdf

Eilstein J, Grégoire S, Fabre A, Arbey E, Géniès C, Duplan H, Rothe H, Ellison C, Cubberley R, Schepky A, Lange D, Klaric M, Hewitt NJ, Jacques-Jamin C (2020). Use of human liver and EpiSkin™ S9 subcellular fractions as a screening assays to compare the *in vitro* hepatic and dermal metabolism of 47 cosmetics-relevant chemicals. J Appl Toxicol. 2020 Jan 8. doi: 10.1002/jat.3914. [Epub ahead of print]

Eisenbrandt DL, Allen SL, Berry PH, Classen W, Bury D, Mellert W, Millischer RJ, Schuh W, Bontinck WJ (1994). Evaluation of the neurotoxic potential of chemicals in animals. Food Chem Toxicol. 32(7):655-69

European Commission (Directorate-General for the Environment), DTU National Food Institute Denmark; Brunel University London, 2017. Supporting the organisation of a workshop on thyroid disruption – Final Report (Framework Contract ENV.A.3/FRA/2014/0029 on implementation of the Community strategy on Endocrine Disrupters). In. Publications Office of the European Union, Luxembourgpp. Available online: https://doi.org/10.2779/921523

Fan X, & Wu, L. (2016). The impact of thyroid abnormalities during pregnancy on subsequent neuropsychological development of the offspring: a meta-analysis. Journal of Maternal-Fetal & Neonatal Medicine, 29(24), 3971-3976. doi:10.3109/14767058.2016.1152248

Fawcett DM & Kirkwood S (1953). The mechanism of the antithyroid action of iodide ion and of the "aromatic" thyroid inhibitors. J. Biol. Chem. 204: 787-796.

Ferrand M, Le Fourn V, Franc JL. (2003). Increasing diversity of human thyroperoxidase generated by alternative splicing. Characterized by molecular cloning of new transcripts with single- and multispliced mrnas. J Biol Chem 278:3793-3800.10.1074/jbc.M209513200

Fini JB, Demeneix B. (2019). [Thyroid disruptors and their consequences on brain development and behavior]. In French. Biol Aujourdhui 213:17-26.10.1051/jbio/2019009

Finken MJJ, van Eijsden, M., Loomans, E. M., Vrijkotte, T. G. M., & Rotteveel, J. (2013). Maternal hypothyroxinemia in early pregnancy predicts reduced performance in

reaction time tests in 5- to 6-year-old offspring. Journal of Clinical Endocrinology and Metabolism, 98(4), 1417-1426. doi:10.1210/jc.2012-3389

Flickinger CW. (1976). The benzenediols: Catechol, resorcinol and hydroquinone--a review of the industrial toxicology and current industrial exposure limits. Am Ind Hyg Assoc J 37:596-606.10.1080/0002889768507526

Flynn JR (2009). Requiem for nutrition as the cause of IQ gains: Raven's gains in Britain 1938–2008. Economics & Human Biology. 7 (1): 18–27. doi:10.1016/j.ehb.2009.01.009. PMID 19251490.

Gaitan E, Merino H, Rodriguez G, Medina P, Meyer JD, DeRouen TA, MacLennan R (1978). Epidemiology of endemic goitre in western colombia. Bull World Health Organ 56:403-416

Gaitan E. (1983). Endemic goiter in western Colombia. Ecol Dis 2:295-308

Gaitan E, Jolley RL, Lindsay RH, Cooksey RC, Hill JB, Island DP. (1987). Resorcinol: Final goitrogenic product in water from a goitrogenic well. Clinical Ecology 5:176-184

Gaitan E, Cooksey, RC and Legan, J (1995). Resorcinol effect on iodide uptake in frtl-5 thyroid cells. . J Investig Med 43:31A

Gardas A, Lewartowska A, Sutton BJ, Pasieka Z, McGregor AM, Banga JP. (1997). Human thyroid peroxidase (tpo) isoforms, tpo-1 and tpo-2: Analysis of protein expression in graves' thyroid tissue1. The Journal of Clinical Endocrinology & Metabolism 82:3752-3757.10.1210/jcem.82.11.4335

Garmendia Madariaga A, Santos Palacios S, Guillén-Grima F, Galofré JC (2014). The Incidence and Prevalence of Thyroid Dysfunction in Europe: A Meta-Analysis, The Journal of Clinical Endocrinology & Metabolism 99(3), 923–931 https://doi.org/10.1210/jc.2013-2409

Garton GA, Williams RT. (1949). Studies in detoxication. 21. The fates of quinol and resorcinol in the rabbit in relation to the metabolism of benzene. The Biochemical journal 44:234-238

Gayrard V, Lacroix MZ, Collet SH, Viguie C, Bousquet-Melou A, Toutain PL, Picard-Hagen N (2013). High bioavailability of bisphenol a from sublingual exposure. Environ Health Perspect 121:951-956.10.1289/ehp.1206339

Géniès C, Jamin EL, Debrauwer L, Zalko D, Person EN, Eilstein J, Grégoire S, Schepky A, Lange D, Ellison C, Roe A, Salhi S, Cubberley R, Hewitt NJ, Rothe H, Klaric M, Duplan H, Jacques-Jamin C (2019a). Comparison of the metabolism of 10 chemicals in human and pig skin explants. J Appl Toxicol 39:385-397.10.1002/jat.3730

Géniès C, Jacques-Jamin C, Duplan H, Rothe H, Ellison C, Cubberley R, Schepky A, Lange D, Klaric M, Hewitt NJ, Grégoire S, Arbey E, Fabre A, Eilstein J (2019b). Comparison of the metabolism of 10 cosmetics-relevant chemicals in EpiSkin™ S9 subcellular fractions and *in vitro* human skin explants. J Appl Toxicol. 2019 Nov 7. doi: 10.1002/jat.3905. [Epub ahead of print]

Gerard AC, Poncin S, Caetano B, Sonveaux P, Audinot JN, Feron O, Colin IM, Soncin F (2008). Iodine deficiency induces a thyroid stimulating hormone-independent early phase of microvascular reshaping in the thyroid. Am J Pathol. 172(3):748-60. doi: 10.2353/ajpath.2008.070841.

Ghassabian A, Marroun, H. E., Peeters, R. P., Jaddoe, V. W., Hofman, A., Verhulst, F. C., White, T. (2014). Downstream effects of maternal hypothyroxinemia in early pregnancy: nonverbal IQ and brain morphology in school-age children. Journal of

Clinical Endocrinology and Metabolism, 99(7), 2383-2390. doi:10.1210/jc.2013-4281

Ghisari M, Bonefeld-Jorgensen EC. (2009). Effects of plasticizers and their mixtures on estrogen receptor and thyroid hormone functions. Toxicol Lett 189:67-77.10.1016/j.toxlet.2009.05.004

Gilbert ME, Rovet J, Chen Z, Koibuchi N. (2012). Developmental thyroid hormone disruption: Prevalence, environmental contaminants and neurodevelopmental consequences. Neurotoxicology 33:842-852.https://doi.org/10.1016/j.neuro.2011.11.005

Glinoer D, De Nayer P, Bourdoux P, Lemone M, Robyn C, Van Steirteghem A, Kinthaert J, Lejeune B (1990). Regulation of Maternal Thyroid during Pregnancy, The Journal of Clinical Endocrinology & Metabolism, 71(2): 276-287, https://doi.org/10.1210/jcem-71-2-276

Grau G, Aguayo, A., Vela, A., Aniel-Quiroga, A., Espada, M., Miranda, G., Rica, I. (2015). Normal intellectual development in children born from women with hypothyroxinemia during their pregnancy. Journal of Trace Elements in Medicine and Biology, 31, 18-24. doi:10.1016/j.jtemb.2015.02.004

Groeneweg S, van Geest FS, Peeters RP, Heuer H, Visser WE (2020). Thyroid Hormone Transporters. Endocr Rev. 41(2). pii: bnz008. doi:10.1210/endrev/bnz008.

Guinet P, Tourniaire J, Peyrin JO. (1967). Clinical and biological study of goiter caused by resorcin. Ann Endocrinol (Paris) 28:199-206

Gyllenberg D, Sourander, A., Surcel, H. M., Hinkka-Yli-Salomäki, S., McKeague, I. W., & Brown, A. S. (2016). Hypothyroxinemia during gestation and offspring schizophrenia in a national birth cohort. Biological Psychiatry, 79(12), 962-970. doi:10.1016/j.biopsych.2015.06.014

Hales C, Taylor, P. N., Channon, S., Paradice, R., McEwan, K., Zhang, L, Ludgate, M. (2018). Controlled Antenatal Thyroid Screening II: effect of treating maternal sub-optimal thyroid function on child cognition. Journal of Clinical Endocrinology and Metabolism. doi:10.1210/jc.2017-02378

Hart FD, Maclagan NF. (1951). Myxœdema from resorcinol ointment. The Lancet 257:530-531.10.1016/S0140-6736(51)92010-7

HEAL. (2014). Health costs in the European Union - how much is related to EDCs? Health and Environment Alliance (HEAL). http://env-health.org/IMG/pdf/18062014_final_health_costs_in_the_european_union_how_much_is_realted_to_edcs-2.pdf

Henrichs J, Bongers-Schokking, J. J., Schenk, J. J., Ghassabian, A., Schmidt, H. G., Visser, T. J., Tiemeier, H. (2010). Maternal thyroid function during early pregnancy and cognitive functioning in early childhood: the Generation R Study. Journal of Clinical Endocrinology and Metabolism, 95(9), 4227-4234. doi:10.1210/jc.2010-0415

Henrichs J, Ghassabian A, Peeters RP, Tiemeier H. (2013). Maternal hypothyroxinemia and effects on cognitive functioning in childhood: How and why? Clin Endocrinol (Oxf) 79:152-162.10.1111/cen.12227

Hewitt NJ, Grégoire S, Cubberley R, Duplan H, Eilstein J, Ellison C, Lester C, Fabian E, Fernandez J, Géniès C, Jacques-Jamin C, Klaric M, Rothe H, Sorrell I, Lange D, Schepky A (2020). Measurement of the penetration of 56 cosmetic relevant chemicals into and through human skin using a standardized protocol. J Appl Toxicol. 2019 Dec 22. doi: 10.1002/jat.3913. [Epub ahead of print]

Hobson QJG. (1951). Varicose ulceration of the legs and myxœdema and goitre following application of resorcinol ointment. J R Soc Med 44:164-166.10.1177/003591575104400222

Howdeshell KL. 2002. A model of the development of the brain as a construct of the thyroid system. Environ Health Perspect 110 Suppl 3:337-348.

Howie RN, Durham EL, Black L, Bennfors G, Parsons TE, Elsalanty ME, Yu JC, Weinberg SM, Cray JJ Jr (2016). Effects of in utero thyroxine exposure on murine cranial suture growth. PLoS One 11:e0167805.10.1371/journal.pone.0167805

Janssen ST & Janssen OE. (2017) Directional thyroid hormone distribution via the blood stream to target sites. Mol Cell Endocrinol. 458:16-21. doi: 10.1016/j.mce.2017.02.037

Jarque S, Fetter E, Veneman WJ, Spaink HP, Peravali R, Strahle U, Scholz S (2018). An automated screening method for detecting compounds with goitrogenic activity using transgenic zebrafish embryos. PLoS One 13:e0203087.10.1371/journal.pone.0203087

Jomaa B, De Haan LHJ, Peijnenburg AACM, Bovee TFH, Aarts JMMJG, Rietjens IMCM. (2015). Simple and rapid *in vitro* assay for detecting human thyroid peroxidase disruption. Altex 32:191-200.10.14573/altex.1412201

Jordan D, Rousset B, Perrin F, Fournier M, Orgiazzi J. (1980). Evidence for circadian variations in serum thyrotropin, 3,5,3′-triiodothyronine, and thyroxine in the rat*. Endocrinology 107:1245-1248.10.1210/endo-107-4-1245

JRC (2013). Key Scientific issues relevant to the identification and characterisation of endocrine disrupting substances – Report of the Endocrine Disruptors Expert Advisory Group (ED EAG). Eds. Munn S. and Gourmenou M. Pp 32. Available at: https://ec.europa.eu/jrc/en/publication/eur-scientific-and-technical-research-reports/key-scientific-issues-relevant-identification-and-characterisation-endocrine-disrupting

Jugan M, Levi Y, Blondeau J. 2010. Endocrine disruptors and thyroid hormone physiology. Biochemical Pharmacology. 79:939-947.

Júlvez J, Álvarez-Pedrerol M, Rebagliato M, Murcia M, Forns J, Garcia-Esteban R, . Lertxundi N, Espada M, Tardón A, Riaño Galán I, Sunyer J (2013). Thyroxine levels during pregnancy in healthy women and early child neurodevelopment. Epidemiology, 24(1), 150-157. doi:10.1097/EDE.0b013e318276ccd3

Katin MJ, Teehan BP, Sigler MH. (1977). Resorcinol induced hypothyroidism in a patient on chronic hemodialysis. Ann Intern Med 86:447-449.10.7326/0003-4819-86-4-447

Kesterke MJ, Judd MA, Mooney MP, Siegel MI, Elsalanty M, Howie RN, Weinberg SM, Cray JJ (2018). Maternal environment and craniofacial growth: Geometric morphometric analysis of mandibular shape changes with in utero thyroxine overexposure in mice. J Anat 233:46-54.10.1111/joa.12810

Kim YC, Matthews HB. (1987). Comparative metabolism and excretion of resorcinol in male and female f344 rats. Fundam Appl Toxicol 9:409-414.10.1016/0272-0590(87)90023-6

Klein FR, Ottis V, Velvart J. (1950). Myxœdema from resorcinol ointment applied to leg ulcers. The Lancet 256:768.10.1016/S0140-6736(50)91705-3

Klem A (1930). Acute medicinal resorcinol poisoning. Norsk. Mag. Laegev. 91:849-850

Kooistra L, Crawford, S., van Baar, A. L., Brouwers, E., & Pop, V. (2006). Neonatal effects of maternal hypothyroxinemia during early pregnancy. Pediatrics, 117(1), 161-167.

Korevaar TIM, Muetzel, R., Medici, M., Chaker, L., Jaddoe, V. W. V., de Rijke, Y. B., Peeters, R. P. (2016). Association of maternal thyroid function during early pregnancy with offspring IQ and brain morphology in childhood: a population-based prospective cohort study. The Lancet Diabetes & Endocrinology, 4(1), 35-43. doi:10.1016/s2213-8587(15)00327-7

Kortenkamp A, Martin O, Faust M, Evans R, McKinlay R, Orton F, Rosivatz E (2012) State of the art assessment of endocrine disrupters. Final report. Available at http://ec.europa.eu/environment/chemicals/endocrine/pdf/sota_edc_final_report.pdf

Krueger T, Long M, Bonefeld-Jørgensen EC. (2008). Plastic components affect the activation of the aryl hydrocarbon and the androgen receptor. Toxicology 246:112-123.10.1016/j.tox.2007.12.028

LaFranchi SH, Haddow JE, Hollowell JG. (2005). Is thyroid inadequacy during gestation a risk factor for adverse pregnancy and developmental outcomes? Thyroid 15:60-71.10.1089/thy.2005.15.60

Larsen PW, Davies TF. Hypothyroidism and thyroiditis. In: Larsen PR, Kronenberg HM, Melmed D, Polonsky KS, editors. Williams textbook of endocrinology. 10 ed. Philadelphia: W. B. Saunders; 2003. p. 415-65.

Lavado-Autric R, Auso E, Garcia-Velasco JV, Arufe Mdel C, Escobar del Rey F, Berbel P, Morreale de Escobar G (2003). Early maternal hypothyroxinemia alters histogenesis and cerebral cortex cytoarchitecture of the progeny. J Clin Invest 111:1073-1082.10.1172/jci16262

Le Fourn V, Ferrand M, Franc JL. (2004) Differential expression of thyroperoxidase mRNA splice variants in human thyroid tumors. Biochim Biophys Acta. un 28;1689(2):134-41.

Leitch VD, Bassett JHD & Williams GR. (2020). Role of thyroid hormones in craniofacial development. Nat Rev Endocrinol 16, 147–164. https://doi.org/10.1038/s41574-019-0304-5

Leese GP, Flynn RV, Jung RT, Macdonald TM, Murphy MJ & Morris AD. (2008). Increasing prevalence and incidence of thyroid disease in Tayside, Scotland: the Thyroid Epidemiology Audit and Research Study (TEARS). Clinical Endocrinology 68 311–316. doi:10.1111/j.1365-2265.2007.03051.x

Leonard JA, Tan YM, Gilbert M, Isaacs K, El-Masri H. (2016). Estimating margin of exposure to thyroid peroxidase inhibitors using high-throughput *in vitro* data, high-throughput exposure modeling, and physiologically based pharmacokinetic/pharmacodynamic modeling. Toxicol Sci 151:57-70.10.1093/toxsci/kfw022

Li Y, Shan, Z., Teng, W., Yu, X., Li, Y., Fan, C., Hua, T. (2010). Abnormalities of maternal thyroid function during pregnancy affect neuropsychological development of their children at 25-30 months. Clinical Endocrinology, 72, 825-829. doi:10.1111/j.1365-2265.2009.03743.x

Lindsay RH, Hill JB, Gaitan E, Cooksey RC, Jolley RL. (1992). Antithyroid effects of coal-derived pollutants. J Toxicol Environ Health 37:467-481.10.1080/15287399209531686

Lope V, Perez-Gomez B, Aragones N, Lopez-Abente G, Gustavsson P, Plato N, Silva-Mato A, Pollán M (2009). Occupational exposure to chemicals and risk of thyroid cancer in Sweden. Int Arch Occup Environ Health 82:267-274.10.1007/s00420-008-0314-4

López-Muñoz E, Mateos-Sánchez L, Mejía-Terrazas GE, Bedwell-Cordero SE (2019). Hypothyroidism and isolated hypothyroxinemia in pregnancy, from physiology to the clinic. Taiwan J Obstet Gynecol. 58(6):757-763. doi:10.1016/j.tjog.2019.09.005.

Lynch BS, Delzell ES, Bechtel DH (2002). Toxicology review and risk assessment of resorcinol: thyroid effects. Regul Toxicol Pharmacol. 36(2):198-210.

Merker PC, Yeung D, Doughty D, Nacht S. (1982). Pharmacokinetics of resorcinol in the rat. Res Commun Chem Pathol Pharmacol 38:367-388

Min H, Dong J, Wang Y, Wang Y, Teng W, Xi Q, Chen J (2016). Maternal hypothyroxinemia-induced neurodevelopmental impairments in the progeny. Mol Neurobiol 53:1613-1624.10.1007/s12035-015-9101-x

Mitchell AL, Hickey B, Hickey JL & Pearce SH (2009). Trends in thyroid hormone prescribing and consumption in the UK. BMC Public Health. 9 132. doi:10.1186/1471-2458-9-132

Modesto T., Tiemeier, H., Peeters, R. P., Jaddoe, V. W., Hofman, A., Verhulst, F. C., & Ghassabian, A. (2015). Maternal mild thyroid hormone insufficiency in early pregnancy and attention-deficit/hyperactivity disorder symptoms in children. JAMA Pediatrics, 169(9), 838-845. doi:10.1001/jamapediatrics.2015.0498

Morreale de Escobar G, Obregon MJ, Escobar del Rey F. (2000). Is neuropsychological development related to maternal hypothyroidism or to maternal hypothyroxinemia? J Clin Endocrinol Metab 85:3975-3987.10.1210/jcem.85.11.6961

Mortoglou A, Candiloros H. The serum triiodothyronine to thyroxine (T3/T4) ratio in various thyroid disorders and after Levothyroxine replacement therapy. Hormones (Athens). 2004 Apr-Jun;3(2):120-6

Motonaga K, Ota M, Odawara K, Saito S, Welsch F. (2016). A comparison of potency differences among thyroid peroxidase (tpo) inhibitors to induce developmental toxicity and other thyroid gland-linked toxicities in humans and rats. Regul Toxicol Pharmacol 80:283-290.10.1016/j.yrtph.2016.06.019

Mughal BB, Fini JB, Demeneix BA (2018). Thyroid-disrupting chemicals and brain development: an update. Endocr Connect ;7(4):R160-R186. doi:10.1530/EC-18-0029.

Narumi S, Fox LA, Fukudome K, Sakaguchi Z, Sugisawa C, Abe K, Kameyama K, Hasegawa T (2017). Mild thyroid peroxidase deficiency caused by tpo mutations with residual activity: Correlation between clinical phenotypes and enzymatic activity. Endocr J 64:1087-1097.10.1507/endocrj.EJ17-0194

Noten A. M., Loomans, E. M., Vrijkotte, T. G., van de Ven, P. M., van Trotsenburg, A. S., Rotteveel, J., Finken, M. J. (2015). Maternal hypothyroxinaemia in early pregnancy and school performance in 5-year-old offspring. European Journal of Endocrinology, 173(5), 563-571. doi:10.1530/EJE-15-0397

NTP. (1992).). National Toxicology Program (NTP). Technical Report on the Toxicology and Carcinogenesis Studies of Resorcinol (CAS No. 108-46-3) in F344/N Rats and B6C3F1 Mice. Gavage Studies, NIH Publication No. 91-2858 Technical report TR403. Testing laboratory: NIH. Report no.: TR403. Owner company: U. S.

Department of Health and Human Services, Public Health Service, National Institute of Health. Report date:1992-07-01Report date:1992-07-01

OECD (2018). Revised guidance document on standardised test guidelines for evaluating chemicals for endocrine disruption. OECD Environment, Health and Safety Publications Series on Testing and Assessment. No. 150. http://www.oecd.org/publications/guidance-document-on-standardised-test-guidelines-for-evaluating-chemicals-for-endocrine-disruption-2nd-edition-9789264304741-en.htm

Oostenbroek M. H. W., Kersten, R. H. J., Tros, B., Kunst, A. E., Vrijkotte, T. G. M., & Finken, M. J. J. (2017). Maternal hypothyroxinaemia in early pregnancy and problem behavior in 5-year-old offspring. Psychoneuroendocrinology, 81, 29-35.

Päkkilä F, Männistö T, Hartikainen AL, Ruokonen A, Surcel HM, Bloigu A, Vääräsmäki M, Järvelin MR, Moilanen I, Suvanto E (2015). Maternal and child's thyroid function and child's intellect and scholastic performance. Thyroid, 25(12), 1363-1374. doi:10.1787/9789264091450-e

Paul KB, Hedge JM, Rotroff DM, Hornung MW, Crofton KM, Simmons SO. (2014). Development of a thyroperoxidase inhibition assay for high-throughput screening. Chem Res Toxicol 27:387-399.10.1021/tx400310w

Paul Friedman KP, Watt ED, Hornung MW, Hedge JM, Judson RS, Crofton KM, Houck KA, Simmons SO (2016). Tiered high-throughput screening approach to identify thyroperoxidase inhibitors within the toxcast phase i and ii chemical libraries. Toxicol Sci 151:160-180.10.1093/toxsci/kfw034

Pedersen M. (2019). Chronic fatigue syndrome and chronic pain conditions - vitally protective systems gone wrong. Scandinavian journal of pain.10.1515/sjpain-2019-0072

Peeters RP, Visser TJ. Metabolism of Thyroid Hormone. [Updated 2017 Jan 1]. In: Feingold KR, Anawalt B, Boyce A, *et al*., editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK285545/

Pop VJ, Kuijpens, J. L., van Baar, A. L., Verkerk, G., van Son, M. M., de Vijlder, J. J., .Vader, H. L. (1999). Low maternal free thyroxine concentrations during early pregnancy are associated with impaired psychomotor development in infancy. Clinical Endocrinology, 50, 149-155.

Pop VJ, Brouwers EP, Vader HL, Vulsma T, van Baar AL, de Vijlder JJ. (2003). Maternal hypothyroxinaemia during early pregnancy and subsequent child development: A 3-year follow-up study. Clin Endocrinol (Oxf) 59:282-288.10.1046/j.1365-2265.2003.01822.x

Porras SP, Hartonen M, Ylinen K, Tornaeus J, Tuomi T, Santonen T. (2018). Environmental and occupational exposure to resorcinol in Finland. Toxicol Lett 298:125-133.10.1016/j.toxlet.2018.03.027

Rayman MP, Bath SC (2015). The new emergence of iodine deficiency in the UK: consequences for child neurodevelopment. Annals of Clinical Biochemistry. 52 705–708. (10.1177/0004563215597249)

Rijk I, van Duursen M, van den Berg M. (2016) Health cost that may be associated with Endocrine Disrupting Chemicals An inventory, evaluation and way forward to assess the potential socio-economic impact of EDC-associated health effects in the EU. IRAS, https://www.uu.nl/sites/default/files/rijk_et_al_2016_-_report_iras_-_health_cost_associated_with_edcs_3.pdf

Roberts MS, Anderson RA, Swarbrick J. (1977). Permeability of human epidermis to phenolic compounds. J Pharm Pharmacol 29:677-683.10.1111/j.2042-7158.1977.tb11434.x

Roberts FP, Wright AL, O'Hagan SA. (1990). Hypothyroidism in textile workers. Occup Med 40:153-156.10.1093/occmed/40.4.153

Roberts CG, Ladenson PW (2004). Hypothyroidism. Lancet. 6;363(9411):793-803.

Román G. C., Ghassabian, A., Bongers-Schokking, J., Jaddoe, V. W. V., Hofman, A., de Rijke, Y. B., Tiemeier, H. (2013). Association of gestational maternal hypothyroxinemia and increased autism risk. Annals of Neurology, 74(5), 733-742. doi:10.1002/ana.23976

Rolaki A, Pistollato F, Munn S, Bal-Price A. (2019). Adverse Outcome Pathway on inhibition of Na+/I- symporter (NIS) leads to learning and memory impairment. OECD Series on Adverse Outcome Pathways n° 14, Éditions OCDE, Paris, doi: https://doi.org/10.1787/7ca86a34-en

Samuel KC. (1955). Experimental production of goiter in rats: With resorcinol and its derivatives. Lab Invest 4:90-105

Santini F, Vitti P, Ceccarini G, Mammoli C, Rosellini V, Pelosini C, Marsili A, Tonacchera M, Agretti P, Santoni T, Chiovato L, Pinchera A (2003). *In vitro* assay of thyroid disruptors affecting tsh-stimulated adenylate cyclase activity. J Endocrinol Invest 26:950-955.10.1007/BF03348190

SCCP (2008). SCCP (Scientific Committee on Consumer Products), Opinion on Resorcinol, 15 April 2008, SCCP/1117/07. http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_124.pdf

Schuebel J, Feldkamp J, Bergmann A, Drossard W, Voigt K. (2017). Latent hypothyroidism in adults. Dtsch Arztebl Int 114:430-438.10.3238/arztebl.2017.430

Seffner W, Schiller F, Heinze R, Breng R. (1995). Subchronic application of humic acids and associated compounds provokes histological changes of goitre in the rat. Exp Toxicol Pathol 47:63-70.10.1016/S0940-2993(11)80288-5

Segal D, Makris SL, Kraft AD, Bale AS, Fox J, Gilbert M, Bergfelt DR, Raffaele KC, Blain RB, Fedak KM, Selgrade MK, Crofton KM. (2015). Evaluation of the ToxRTool's ability to rate the reliability of toxicological data for human health hazard assessments. Regul Toxicol Pharmacol. 72(1):94-101

Sharlin DS, Gilbert ME, Taylor MA, Ferguson DC, Zoeller RT. (2010). The nature of the compensatory response to low thyroid hormone in the developing brain. J Neuroendocrinol 22:153-165.10.1111/j.1365-2826.2009.01947.x

Singh R, Al Khalili Y. Benzocaine. [Updated 2020 Mar 2]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK541053/

Soldin OP. (2006). Thyroid function testing in pregnancy and thyroid disease: Trimester-specific reference intervals. Ther Drug Monit 28:8-11.10.1097/01.ftd.0000194498.32398.7b

Stagnaro-Green A, Abalovich M, Alexander E, Azizi F, Mestman J, Negro R, Nixon A, Pearce EN, Soldin OP, Sullivan S, Wiersinga W; American Thyroid Association Taskforce on Thyroid Disease During Pregnancy and Postpartum (2011) Guidelines of the American Thyroid Association for the diagnosis and management of thyroid disease during pregnancy and postpartum. Thyroid 21(10):1081–1125. doi:10.1089/thy.2011.0087

Steinmaus C, Pearl M, Kharrazi M, Blount BC, Miller MD, Pearce EN, Valentin-Blasini L, DeLorenze G, Hoofnagle AN, Liaw J (2016). Thyroid hormones and moderate exposure to perchlorate during pregnancy in women in southern California. Environmental Health Perspectives. 124; 861–867. (10.1289/ehp.1409614)

Stenbäck F, Shubik P. (1974). Lack of toxicity and carcinogenicity of some commonly used cutaneous agents. Toxicol Appl Pharmacol 30:7-13.10.1016/0041-008X(74)90242-7

Stenbäck F. (1977). Local and systemic effects of commonly used cutaneous agents: Lifetime studies of 16 compounds in mice and rabbits. Acta Pharmacol Toxicol (Copenh) 41:417-431.10.1111/j.1600-0773.1977.tb02152.x

Strakosch E (1943). Studies on ointments? V. Ointments containing resorcinol. Arch. Dermatol. Syphilol. 48:393-399

Takemura Y, Yamada T, Ozawa K, Shichijo K. (1966). Comparison of the effects of several drugs on tissue uptake of labeled thyroxine and triiodothyronine in the presence of rat plasma *in vitro*. Metabolism 15:679-686.10.1016/s0026-0495(66)80003-3

Taurog A. 1970. Thyroid peroxidase and thyroxine biosynthesis. In: Proceedings of the 1969 laurentian hormone conference, Vol. 26, (Astwood EB, ed). Boston:Academic Press, 189-247.

Teasdale TW, Owen DR (2005). A long-term rise and recent decline in intelligence test performance: The Flynn Effect in reverse. Personality and Individual Differences. 39 (4): 837–43. doi:10.1016/j.paid.2005.01.029.

Teasdale TW, Owen DR (2008). Secular declines in cognitive test scores: A reversal of the Flynn Effect (PDF). Intelligence. 36 (2): 12126. doi:10.1016/j.intell.2007.01.007.

Thienpont B., Tingaud-Sequeira A., Prats E., Barata C., Babin P.J. & Raldua D. (2011). Zebrafish Eleutheroembryos Provide a Suitable Vertebrate Model for Screening Chemicals that Impair Thyroid Hormone Synthesis (including Supporting Information). Environ. Sci. Technol. 45(17): 7525-32.

Thomas AE, Gisburn MA. (1961). Exogenous ochronosis and myxoedema from resorcinol. Br J Dermatol 73:378-381

Thompson W., Russell, G., Baragwanath, G., Matthews, J., Vaidya, B., & Thompson-Coon, J. (2018). Maternal thyroid hormone insufficiency during pregnancy and risk of neurodevelopmental disorders in offspring: A systematic review and meta-analysis. Clinical Endocrinology.

TOMA (1978) 1978 cross-sectional health study of workers at the Petrolia, Pennsylvania, plant of Koppers Company Inc. Final report. Tabershaw Occupational Medicine Associates (unpublished) [cited in Delzell, 2000].

TOMA (1981) Occupational health evaluation of the Petrolia, Pennsylvania, plant of Koppers Company Inc. Final report. Tabershaw Occupational Medicine Associates (unpublished) [cited in Delzell, 2000].

Trasande L, Zoeller RT, Hass U, Kortenkamp A, Grandjean P, Myers JP, DiGangi J, Hunt PM, Rudel R, Sathyanarayana S, Bellanger M, Hauser R, Legler J, Skakkebaek NE, Heindel JJ (2016). Burden of disease and costs of exposure to endocrine disrupting chemicals in the European Union: an updated analysis. Andrology 4(4):565–572, PMID: 27003928, 10.1111/andr.12178

Tsomi V, Kalopissis G. (1982). Cutaneous penetration of some hairdyes in the hairless rat. Toxicological European Research 4:119-127

Tukes - Finnish Safety and Chemicals Agency (2017). Substance Evaluation Conclusion as required by REACH Article 48 and Evaluation Report for Resorcinol, EC No 203-585-2, CAS No 108-46-3. Evaluating Member State(s): Finland. 24 October 2017 https://echa.europa.eu/documents/10162/fedfa3b0-f8a2-66b4-2a08-7f686df46994

Unpublished study report (1977). A ninety-day inhalation exposure to resorcinol. Public information available on ECHA dissemination site https://echa.europa.eu/registration-dossier/-/registered-dossier/13740/7/6/3

Unpublished study report (1982a). Resorcin : oral (gavage) teratology study in the rat.

Unpublished study report (1982b). Resorcin : oral (gavage) teratology study in the New Zealand white rabbit. Public information available on ECHA dissemination site https://echa.europa.eu/registration-dossier/-/registered-dossier/13740/7/9/3/?documentUUID=0c5a02d5-0c23-4e86-baa1-8401d6631e1e

Unpublished study report (1999). *In vitro* studies for evaluating estrogenic and anti-estrogenic activities of resorcinol.

Unpublished study report (2003). A drinking water dose range-finding reproductive toxicity study of resorcinol in rats.

Unpublished study report (2004a). 13-week study by oral route (gavage) in rats followed by a 4-week treatment-free period. Public information available on ECHA dissemination site https://echa.europa.eu/registration-dossier/-/registered-dossier/13740/7/6/2

Unpublished study report (2004b). Prenatal developmental toxicity study by oral route (gavage) in rats. Public information available on ECHA dissemination site https://echa.europa.eu/registration-dossier/-/registered-dossier/13740/7/9/3

Unpublished study report (2005a). A drinking water two-generation reproductive toxicity study of resorcinol in rats. Public information available on ECHA dissemination site https://echa.europa.eu/registration-dossier/-/registered-dossier/13740/7/9/2

Unpublished study report (2005b). No information on report name. [Quoted from Tukes, 2017]. Public information available on ECHA dissemination site https://echa.europa.eu/registration-dossier/-/registered-dossier/13740/7/2/3

US EPA. United States Environmental Protection Agency (2019). Proposed Approaches to Inform the Derivation of a Maximum Contaminant Level Goal for Perchlorate in Drinking Water. Volume I. May 2019

van Mil NH, Steegers-Theunissen RPM, Bongers-Schokking JJ, El Marroun H, Ghassabian A, Hofman A, Jaddoe VW, Visser TJ, Verhulst FC, de Rijke YB, Steegers EA, Tiemeier H (2012). Maternal hypothryoxinemia during pregnancy and growth of the fetal and infant head. Reproductive Sciences, 19(12), 1315-1322. doi:10.1177/1933719112450338

Vandenberg LN, Welshons WV, Vom Saal FS, Toutain P-L, Myers JP. (2014). Should oral gavage be abandoned in toxicity testing of endocrine disruptors? Environmental health : a global access science source 13:46-46.10.1186/1476-069X-13-46

Wang P, Gao, J., Zhao, S., Guo, Y., Wang, Z., & Qi, F. (2016). Maternal thyroxine levels during pregnancy and outcomes of cognitive development in children. Molecular Neurobiology, 53(4), 2241-2248. doi:10.1007/s12035-015-9189-

Waring RH, Ramsden DB, Jarratt PDB, Harris RM. (2012). Biomarkers of endocrine disruption: Cluster analysis of effects of plasticisers on phase 1 and phase 2

metabolism of steroids. Int J Androl 35:415-423.10.1111/j.1365-2605.2012.01248.x

Welsch F, Nemec MD, Lawrence WB. (2008a). Two-generation reproductive toxicity study of resorcinol administered via drinking water to crl:Cd(sd) rats. Int J Toxicol 27:43-57.10.1080/10915810701876679

Welsch F. (2008b). Routes and modes of administration of resorcinol and their relationship to potential manifestations of thyroid gland toxicity in animals and man. Int J Toxicol 27:59-63.10.1080/10915810701876687

Williams Textbook of Endocrinology (Thirteenth Edition) (2016). Editor(s): Shlomo Melmed, Kenneth S. Polonsky, P. Reed Larsen, Henry M. Kronenberg. ISBN 9780323297387, https://doi.org/10.1016/B978-0-323-29738-7.00046-0.

WHO/International Programme on Chemical Safety. (2002) Global assessment of the state of the science of endocrine disruptors (Damstra T, Barlow S, Bergman A, Kavlock R, Van Der Kraak G, eds.) http://www.who.int/ipcs/publications/new_issues/endocrine_disruptors/en/

WHO (2006) Resorcinol. Concise international chemical assessment document (CICADS) 71. World Health Organization http://www.inchem.org/documents/cicads/cicads/cicad71.htm

Wolfram LJ, Maibach HI. (1985). Percutaneous penetration of hair dyes. Archives of Dermatological Research 277:235-241.10.1007/BF00404323

Wondisford FE (2015). A direct role for thyroid hormone in development of the adrenal cortex. Endocrinology, 156(6), 1939–1940. https://doi.org/10.1210/en.2015-1351

Wong EY, Ray R, Gao DL, Wernli KJ, Li W, Fitzgibbons ED, Feng Z, Thomas DB, Checkoway H (2006). Reproductive history, occupational exposures, and thyroid cancer risk among women textile workers in shanghai, china. Int Arch Occup Environ Health 79:251-258.10.1007/s00420-005-0036-9

Yeung D, Kantor S, Nacht S, Gans EH. (1983). Percutaneous absorption, blood levels, and urinary excretion of resorcinol applied topically in humans. Int J Dermatol 22:321-324.10.1111/j.1365-4362.1983.tb02149.x

Zoeller RT & Rovet J (2004). Timing of thyroid hormone action in the developing brain: clinical observations and experimental findings. J Neuroendocrinol. 16(10):809-18.

Zoeller RT, Tyl RW, Tan SW. 2007. Current and Potential Rodent Screens and Tests for Thyroid Toxicants. Critical Reviews in Toxicology 37:55-95.

Annex I – *In vitro* data related to other hormonal systems than thyroid

Estrogenic and anti-estrogenic activities of resorcinol were evaluated using two *in vitro* receptor-mediated assays (Unpublished study report, 1999). A mammalian cell-based luciferase reporter gene assay was developed for detecting estrogenic and anti-estrogenic effects of chemicals on human estrogen receptor α (hERα)-mediated transactivation (n=6).

Effects on ligand-dependent interaction between hERα and a coactivator (TIF2: Transcriptional Intermediary Factor 2) were also examined by a yeast two-hybrid assay (n=4). DMSO was used as solvent. Marked positive effects of 17ß-estradiol (E2) were detected by both assays. Anti-estrogen, 4-hydroxytamoxifen inhibited the E2-mediated transactivation in the luciferase assay. **Neither significant estrogenic nor anti-estrogenic effects of resorcinol were found by the assays performed** on 5 concentrations in the concentration range 1 nM to 10 µM.

Estrogenic activity of resorcinol was tested in estrogen receptor (ER) reporter gene assay (Ghisari *et al.*, 2009) using the stable transfected MVLN cells, a derivative of the ER positive human breast carcinoma MCF-7 cell line carrying the estrogen response element (ERE)-luciferase reporter vector. A concentration range of $1x10^{-10}$ to $1x10^{-5}$ M resorcinol in DMSO was tested in the absence or in the presence of 17ß-estradiol (E2) at its EC_{50} concentration. At least three independent experiments were conducted. **No significant activity was observed for resorcinol.**

The effects of resorcinol on the aryl hydrocarbon receptor (AhR) and the androgen receptor (AR) were assessed using CALUX luciferase reporter gene assays (Krueger *et al*., 2008). Resorcinol was tested in DMSO and over a concentration of $1x10^{-10}$ to $1x10^{-4}$ M. The AhR-CALUX assay was conducted without or with TCDD at its EC_{50}. No agonistic response to AhR was observed for resorcinol but **resorcinol enhanced the TCDD induced AhR activity in a dose-dependent manner**. A significant response ($p<0.05$) was observed from $1x10^{-8}$ M. An effect of 175% was observed at $1x10^{-7}$ M, the maximum response at non-toxic concentration. EC_{50} was $7.6x10^{-5}$ M. Additive effects were observed when tested in a mixture of 6 phenols and plasticisers.

The AR-CALUX assay was conducted in triplicate without or with the AR agonist R1881 at its EC_{50}. No agonistic response to AR was observed for resorcinol. **The effect of R1881 was reduced to 53% at $1x10^{-4}$ M**, the maximum response at non-toxic concentration but none of the tested concentrations were significantly different from controls. Dose-response analysis resulted in an EC_{50} of $2.9x10^{-5}$ M. Additive effects were observed when tested in a mixture of 6 phenols and plasticisers.

Resorcinol was included in the EU-funded ENDOMET project and was tested in 23 different assays related to steroid metabolism and function (Waring *et al.*, 2012). Assays related to thyroid function are described in section 4.5.1. Results of other assays are summarised in the table below. **Resorcinol was detected inactive in the experimental conditions of all assays except for the aromatase inhibition assay, in which a measurable effect was seen at concentration ≤ 10^{-7} M.**

Table I-1 – Assays related to potential ED activities of resorcinol in Waring *et al.* (2012)

Assays		Response under assay conditions
Effect on steroid metabolism	Activity of sulphotransferase 1A1 (from human liver cytosol)	Inactive
	Activity of sulphotransferase 1E1	Inactive
	Activity of sulphotransferase 2A1	Inactive
	Activity of cysteine dioxygenase	Inactive
	Activity of sulphite oxidase (mRNA expression in human neuronal medulloblastoma cell line	Inactive
	Activity of PAPS synthase (mRNA expression in human neuronal medulloblastoma cell line TE671)	Inactive
	Effect on lecting branching	Inactive
	Inhibition of aromatase (human choriocarcinoma cell line JEG-3)	Measurable effect seen at concentration ≤ 10^{-7} M
Interaction with nuclear receptors	ER (human breast cell line MVLN stably transfected with ERE-luciferase reporter vector)	Inactive
	AR	Inactive
	AhR	Inactive
Steroid hormone production in porcine ovarian granulosa cells	Effect on basal progesterone production	Inactive
	Effect on FSH-stimulated progesterone production	Inactive
	Effect on FSH-stimulated estrogen production	Inactive
Brain cell signaling activity in human cell line SK-N-MC	Glycogen Synthase Kinase (GSK)	Inactive
	MAP Kinase	Inactive
	Presence of ROS	Inactive
	Transcriptional activation of GSK	Inactive

Resorcinol was included in the Toxcast phase II chemical libraries of the US EPA screening program and was screened for chemical activity in 665 assays[17]. Resorcinol was concluded active in 24 assays, 6 of them being related to potential disruption of endocrine pathways as summarised in the Table below.

[17] https://comptox.epa.gov/dashboard/dsstoxdb/results?search=DTXSID2021238&abbreviation=TOXCAST_PHASEII#bioactivity, accessed on 19 July 2019

Table I-2 – Toxcast assays related to ED potential with ACTIVE response of resorcinol

RESORCINOL ACTIVE IN TOXCAST ASSAYS				
Toxcast assay name	**AC_{50} (µM)**	**Threshold for positive response**	**Intended target family**	**Assay description**
OT_ERα_EREGFP_0480	54.1	20.0	Nuclear receptor	Expression of human estrogen receptor 1 gene (inducible receptor gene)(cervix cell line HeLa)
OT_ER_ERβERβ_1440	18.0	20.0	Nuclear receptor	Binding to human ERß (kidney cell line HEK293T)
OT_ER_ERαERβ_1440	32.1	20.0	Nuclear receptor	Binding to human ERα and ERß (kidney cell line HEK293T)
ATG_DR5_CIS_up	4.40	0.592	Nuclear receptor	Expression of human RAR (hepatic cell line HepG2)
NCCT_TPO_AUR_dn*	0.0244	20	Oxido-reductase	Activity of TPO enzyme in AUR-TPO assay (rat thyroid cells)
CEETOX_H295R_CORTISOL_dn	22.4	1.01	Steroid hormone	Induction of cortisol (human adrenal cell line H295R)

* see also above Paul Friedman *et al.* 2016

Resorcinol was concluded inactive in 641 assays including 21 assays in the intended target family "steroid hormones" and 158 assays in the intended family "nuclear receptor".