

1 **Title:**

2 **Limits of the regulatory evaluation of resorcinol as a thyroid disruptor: when limited experimental data**  
3 **challenge established effects in humans**

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22 *All authors declare they have nothing to disclose.*

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## 26 **Abstract**

27

28 Severe hypothyroidism has been reported in humans during resorcinol therapeutic use. However, available  
29 data highlight differences in the severity of resorcinol-induced thyroid effects between humans and rodents,  
30 leading to a debate on the relevance of human data for its classification as a thyroid disruptor.

31 The aim of this review is to illustrate some of the limitations of the evaluation framework for thyroid  
32 disrupters using resorcinol as a case study of a chemical with clear thyroid-disrupting properties in humans  
33 that could not have been identified solely from regulatory studies on animals.

34 The reliability of human data has been called into question due to the specific exposure patterns in humans  
35 and the paucity of robust toxicokinetic data. In humans, therapeutic use of resorcinol induces severe  
36 hypothyroidism, but in rodents, thyroid disruption is limited to decreased thyroxine concentrations and  
37 histological changes in the thyroid. The adverse effects of thyroid disruption, such as impaired  
38 neurodevelopment, have not been sufficiently investigated, and experimental neurobehavioral data for  
39 resorcinol remain scarce and inconclusive.

40 Although regulatory toxicological evaluations have not included in-depth investigations of thyroid regulation  
41 and related adverse effects, they have been used to challenge the relevance of human data. Resorcinol is an  
42 emblematic example of how the framework for regulatory evaluations of thyroid disruptors relies almost  
43 exclusively on animal studies which may not be suitable for assessing thyroid disruption. This review  
44 highlights the need to revise regulatory guidelines and to adopt strategies based on up-to-date, scientifically  
45 sound approaches to identify thyroid disruptors. The limits of the current regulatory framework for  
46 identifying thyroid disruptors can lead to opposing positions between regulatory bodies. The French Agency  
47 for Food, Environmental and Occupational Health & Safety (ANSES)'proposal to identify resorcinol as a  
48 "substance of very high concern" due to its ED properties has not been adopted by the European instances.

49

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## 61 Introduction

62

63 Risk assessments of endocrine-disrupting chemicals (EDCs) constitute a huge challenge because the  
64 endocrine system regulates nearly all physiological processes in a timely and often non-monotonic manner.  
65 Regulatory bodies throughout the world are therefore continuously looking for new strategies for the  
66 assessment of these compounds (Barton-Maclaren et al., 2022; Browne et al., 2020). Although the World  
67 Health Organization (WHO)'s definition of EDCs is consensually recognized (WHO, 2002), the existing  
68 institutional framework for chemical evaluation specifically addressing endocrine disruption — whether  
69 based on the Environmental Protection Agency (EPA)'s tiered approach in the United States or on the  
70 Organization for Economic Co-operation and Development (OECD)'s five-level rating approach in the  
71 European Union — remain incomplete and limited to very few guidelines, some of which are obsolete and/or  
72 inappropriate for EDCs. Historically, the focus has been placed on the male and female reproductive  
73 functions. The thyroid endocrine system came to the forefront much later, highlighting major gaps in the  
74 tools available for the hazard assessment of thyroid-disrupting chemicals (TDCs). The thyroid function is  
75 critical for both the regulation of key physiological processes in adult and adequate development. The  
76 amphibian metamorphosis assay, based on the thyroid-dependent metamorphosis process, is used for low-  
77 level (OECD level 2 and 3) or tier (US EPA tier 1) tests to investigate thyroid-specific endpoints (Table 1).  
78 However, even in the amphibian test guidelines, investigation of parameters characterizing the thyroid  
79 function remains extremely limited. In higher vertebrates, there is no specific endpoint such as  
80 metamorphosis that can be considered as diagnostic of thyroid disruption. Further, many adverse effects  
81 potentially associated with exposure to TDCs can result from other modes of action (MoA). Thus, disruption  
82 of such a complex and pluripotent endocrine function is difficult to test with available traditional regulatory  
83 schemes elaborated for highly specialized function such as reproduction. The robust identification of a cause-  
84 effect relationship between a thyroid-related MoA and an adverse effect, which is mandatory for a substance  
85 to be recognized as an EDC targeting the thyroid function, may be very challenging. To overcome such  
86 difficulties, regulatory bodies around the world have encouraged the use of adverse outcome pathway (AOP)  
87 approaches, which link molecular events to adverse effects via a plausible MoA (Browne et al., 2020, 2017),  
88 and/or other approaches based on a broad, holistic view of the mechanistic evidence (La Merrill et al., 2020)  
89 for the identification of EDCs including TDCs (Noyes et al., 2019). Although these approaches have proved  
90 helpful in improving the efficiency of TDC evaluation by allowing the integration of academic data into the  
91 evaluation process, there are only a few reviewed and published AOPs available for TDCs, and these are  
92 limited to neurodevelopmental toxicity endpoints. Despite this clear data gaps, until 2013, no EU-funded,  
93 large-scale project had directly addressed TDC issues. It was only in 2020 that three large-scale EU projects

94 dedicated to the development of innovative and specific methods to detect TDCs were finally launched (the  
95 ATHENA (Kortenkamp et al. 2020), SCREENED and ERGO projects).

96 Despite the peculiarities of the thyroid function and the fact that existing guidelines are clearly insufficient  
97 when considering thyroid disruption, there is currently no guideline exclusively dedicated to TDC evaluation.  
98 For example, evaluating thyroid disruption is barely mentioned in the different levels/tiers of either OECD or  
99 US-EPA frameworks (Noyes et al., 2019). When measurements of thyroid-related parameters are mentioned,  
100 they are rarely mandatory and usually very perfunctory. They are generally limited to a merely descriptive  
101 evaluation of thyroid gland histology and/or thyroid hormone (TH) measurements (Table 1). Guidance has  
102 been produced at the EU level (ECHA/EFSA 2018), emphasizing what should be considered an adverse effect  
103 and how to handle notable differences in the systemic regulation of TH levels between commonly used  
104 experimental animal models and humans. Although there is undoubtedly a desire to improve the framework  
105 of TDC evaluation, major gaps have not been addressed at all, leaving the door wide open to debates on the  
106 relevance of the analysis. There is to date no comprehensive testing strategy or integrated conceptual  
107 framework that can integrate all available data across various fields — from molecular testing to human  
108 clinical observations — or propose decision strategies when some type of data is lacking or insufficiently  
109 documented. An integrated strategy is vital for TDC, because animal models recommended for high level/tier  
110 mammal trials of regulatory evaluation, *i.e.* frequently rodents, can be easily — and are — challenged with  
111 regard to their relevance for human thyroid system regulation. Finally, one of the major caveats of TDC  
112 evaluation arises from the fact that none of the guidelines are designed to establish the link between thyroid  
113 disruption and apical adverse effects, one of the most worrying being the alteration of neurodevelopment.  
114 From this standpoint, identifying and including neurodevelopmental endpoints that could potentially be  
115 more sensitive and/or specific of thyroid disruption e.g. periventricular cortical heterotopia (Gilbert et al.,  
116 2014) could reveal itself to be very helpful. An optimized protocol for thyroid hormone measurements in  
117 dams and pups allowing a better coverage of the window of susceptibility in terms of the thyroid axis  
118 development as well as thyroid-dependent neurodevelopmental processes would undoubtedly constitute a  
119 huge progress.

120 In this context, the evaluation of resorcinol is a striking example of the gaps in TDC evaluation and, in  
121 particular, of how complying strictly and solely with the few recommendations for TDCs in the ED evaluation  
122 conceptual framework can be misleading if the well-established endocrine toxicity in humans is not  
123 considered. Resorcinol (CAS 108-46-3) is a small molecule with a wide range of applications (WHO, 2006):  
124 ultraviolet absorber, antioxidant, antiseptic, etc. In the context of its use as a medicinal product in humans  
125 in particular to treat skin ulcers, resorcinol has long been described to interfere with thyroid function  
126 (Fawcett and Kirkwood, 1953). There is a unique set of experimental and human studies describing its  
127 thyroid-disrupting properties (Supplemental Material, Table1). Human data show severe clinical

128 hypothyroidism with associated goiter. Other than goiter, no other potential apical adverse effect related to  
129 thyroid system disruption has ever been properly investigated. Resorcinol is a prime example of a situation  
130 where there is a clear demonstration of thyroid disruption in humans, but for which *in vivo* experimental data  
131 lead to mitigated conclusions. The evaluation of thyroid function in guideline studies, although complying  
132 with official guidelines, is incomplete and limited.

133

134 Available data on resorcinol, whether from academic or regulatory studies, were analyzed in the context of  
135 the French Agency for Food, Environmental and Occupational Health & Safety (ANSES)'s proposal (ANSES,  
136 2020) to identify resorcinol as a “substance of very high concern” due to its TD properties under the European  
137 Regulation on the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH). The main  
138 objective of this commentary is to use discrepancies between different types of studies, more particularly  
139 human clinical and animal experimental studies, regarding the thyroid disrupting properties of resorcinol, to  
140 highlight some of the limitations of the thyroid disruptor evaluation framework. More specifically,

141 we aimed to shed light on why and how a clear AOP linking a thyroid-related MoA (i.e. thyroid peroxidase  
142 (TPO) inhibition) to adverse effects in humans (clinical hypothyroidism) was not considered reliable enough  
143 to identify resorcinol as a “substance of very high concern” due to its TDC properties (ECHA, 2020). We set  
144 out to gain an understanding of how the limitations of available regulatory data can lead to the failure to  
145 identify TDCs in the absence of human data. Ultimately, our goal is to provide insights for further  
146 improvements to the regulatory framework for TDC evaluation and testing.

147

## 148 **Methods**

149 Our analysis focused on demonstrating the ability of resorcinol to disrupt thyroid function based on the EU  
150 Joint Research Center (JRC)'s application of the WHO definition (WHO, 2002; JRC, 2013): inducing an adverse  
151 effect, having an endocrine MoA, and a plausible link between the adverse effect and the MoA based on  
152 general knowledge or existing and validated AOPs. Here, we considered regulatory studies submitted in the  
153 context of the European REACH regulation. In addition, studies from an exhaustive literature search  
154 conducted until 12 November 2019 were selected for their relevance and adequacy for the assessment of  
155 thyroid disruption, and their reliability was assessed using ToxRtool (Segal et al., 2015). A bibliographic search  
156 regarding the thyroid effects of resorcinol was updated until 26 October 2021. All studies were qualitatively  
157 weighted, based on expert judgment, to analyze the thyroid-disrupting properties of resorcinol. Only data  
158 appropriate for the demonstration of the limitations and pitfalls of the framework for the regulatory  
159 identification of EDs are presented here. Comprehensive detailed data are available in the ANSES's proposal

160 to identify resorcinol as a substance of very high concern due to its TD properties (ANSES, 2020) that has  
161 been published on the European Chemicals Agency (ECHA, 2020) website.

162 Summary tables of human and experimental data are available as Supplemental Material.

163

#### 164 **Discussion**

##### 165 Do animal and human data lead to conflicting conclusions?

166 Ten human cases published between 1950 and 1977 (Supplemental Material, Tables 2–6) reported severe  
167 clinical hypothyroidism as an adverse effect of long-term chronic exposure to resorcinol via the application  
168 of a cutaneous ointment for the treatment of cutaneous ulcers (for review see Lynch et al. 2002). Biological  
169 parameters and thyroid histology, when available, were consistent with a blockage of TH synthesis.  
170 Regression of the goiter and other symptoms after cessation of exposure to resorcinol in 8 of the 10 reported  
171 cases demonstrated the causal relationship between adverse effects of resorcinol in adults and the thyroid  
172 MoA. This reversibility attenuates the perception of adversity. However, this seeming reversibility is quite  
173 misleading, because more subtle subclinical thyroid alterations during developmental stages can lead to  
174 irreversible damages, particularly in terms of neurobehavioral development (Korevaar et al., 2018; Zoeller  
175 and Rovet, 2004). In rodent studies, results have been called into question due to the poor quality of the data  
176 and/or major differences in the experimental designs. The available regulatory studies indeed provide  
177 measurements of all the mandatory thyroid parameters for regulatory toxicology. However, these studies  
178 were too limited for a proper investigation of thyroid function and often did not include the most relevant  
179 period of measurements (no measurement of THs during gestation or concomitant to neurobehavioral tests)  
180 with regard to the only thyroid-relevant apical adverse effect investigated, *i.e.* neurodevelopment.

181 Results obtained via the oral route in rodents differ markedly between gavage and diet or drinking water  
182 administration. Most often, no effect could be demonstrated with gavage, including at very high doses. By  
183 contrast, thyroid parameters were often modified in a sense suggesting decreased thyroid activity following  
184 more “physiological” oral routes, such as the diet or drinking water, including at much lower doses (for detail,  
185 see Supplemental Material, Tables 2–6). Interestingly, thyroid histopathological lesions and enlargement,  
186 two phenomena considered as diagnostic of thyroid disruption by standard-setting organizations such as the  
187 OECD or US EPA (Table 1), have been observed in rats with subcutaneous administration of aqueous  
188 (Cheymol et al., 1951) or oil solutions at doses within the range of the therapeutic dose for humans, and long  
189 durations of exposure (Doniach and Logothetopoulos, 1953; Samuel, 1955). The results of these old pre-  
190 Guideline studies are not precisely described, and it is difficult to determine the significance of these  
191 observations. Similar effects have been observed following dermal applications of ointment at a much higher

192 concentrations for 4 weeks (Samuel, 1955), but not after shorter applications for 3 weeks (Doniach and  
193 Logothetopoulos, 1953).

194 Developmental exposure to resorcinol through drinking water (Unpublished Study report, 2003; Welsch et  
195 al., 2008) was associated with sex, dose and age-dependent changes for some thyroid parameters. Overall,  
196 except for an increased T3 concentration in F0 females and F1 and F2 pups at weaning, all observed  
197 modifications argue in favor of decreased thyroid function: decreased colloid content in F0 males, increased  
198 thyroid-stimulating hormone (TSH) concentration in F1 male offspring at weaning at higher doses, increased  
199 thyroid weight jointly with decreased T4 levels in female F2 pups at weaning. Interestingly, similar patterns  
200 of thyroid alterations have been observed in mildly hypothyroid children with TPO gene mutations, with  
201 mildly elevated serum TSH levels, normal or slightly low serum T4 levels, and a high T3:T4 molar ratio (Narumi  
202 et al., 2017).

203 Overall, the data available for rodents suggest that resorcinol can affect the thyroid function when given  
204 through “physiological” oral or parenteral routes, but not through gavage. Therefore, there are no major  
205 discrepancies between animal and human studies regarding the plausibility of a thyroid MoA of resorcinol.  
206 The possibility remains however that ointment application on damaged skin can lead to overexposure in  
207 human subjects. This potential overexposure has not been documented by measuring resorcinol and/or its  
208 metabolite concentrations in patients. The question remains of whether the route of administration and  
209 associated internal exposure can be determining for the effect of resorcinol on thyroid function in humans.

210 Is the human route of exposure prohibitive?

211 Two toxicokinetic (TK) lines of evidence were used to challenge the regulatory identification of resorcinol as  
212 an endocrine disruptor during European debates on the proposal to identify resorcinol as a substance of very  
213 high concern due to its TD properties (ECHA, 2020). Firstly, gavage data showing no effect of resorcinol on  
214 the thyroid function in rats was considered as strong evidence, assuming that this route is fully representative  
215 of oral exposure, with no further consideration of resorcinol TK properties. Secondly, it was assumed, again  
216 with no further consideration of resorcinol TK properties, that the therapeutic use of resorcinol was  
217 associated with a high level of internal exposure.

218 TK studies conducted in rats and rabbits by gavage showed highly efficient digestive absorption of resorcinol  
219 (from 77% to 95% within 24 h of gavage; Garton and Williams 1949; Kim and Matthews 1987), and extensive  
220 metabolization before urinary excretion. About 90% of the administered dose is recovered after 24 h in urine,  
221 mainly as a glucuronide conjugate (Garton and Williams 1949; Kim and Matthews 1987). *In vitro* results  
222 obtained in human hepatic S9 fractions and primary human hepatocytes (Eilstein et al., 2020) suggest that  
223 the hepatic conjugation of resorcinol is very efficient in humans as well. The high and rapid metabolization



224 rates are consistent with a high hepatic first-pass effect, which would thus limit internal exposure following  
225 gavage. In the case of “physiological” oral routes, the buccal and sublingual mucosae are directly exposed to  
226 the compound. Depending on the physicochemical properties of the compound, this may result in partial  
227 direct absorption from these mucosae, bypassing first-pass metabolic effects similarly to parenteral routes.  
228 Therefore, gavage data may not be ideal for the evaluation of the effect of exposure via the oral route, in  
229 particular for substances that are quickly metabolized in the liver. In addition, the stressful conditions related  
230 to this route might hinder clear conclusions on biological effects including endocrine effects. Indeed, gavage  
231 by itself can induce neuroendocrine modifications that might be stress-related including in the context of  
232 developmental exposure (Cao et al., 2013; McDonnell-Dowling et al., 2017).

233 Thus, the relevance of the effects observed in humans exposed through parenteral route cannot be  
234 challenged solely on the basis of the absence of effect with gavage. Furthermore, more generally, these  
235 considerations regarding administration should prompt regulatory bodies to modify guidelines, making  
236 “physiological” routes the reference administration route in regulatory studies, unless justified by technical  
237 or physicochemical limitations.

238         The main line of evidence for the thyroid-disrupting activity of resorcinol comes from human data in  
239 which resorcinol was applied as an ointment on damaged skin (for review, see ANSES 2020). The relevance  
240 of these data was considered debatable based on the argument that the breakdown of the cutaneous barrier  
241 can lead to increased bioavailability and overexposure in patients. However, no measurements of blood  
242 concentration in these patients are available to test this hypothesis. For intact skin, dermal absorption seems  
243 rather low as shown in human volunteers following discrete, repeated applications of hydroalcoholic  
244 solutions (Yeung et al., 1983), hair dye formulations (Roberts et al., 1977) or *ex vivo* on skin explants. In all  
245 cases, no more than 5% of the daily dose was absorbed after 24 h. These data however contradict more  
246 recent studies on pig and human skin explants, indicating transcutaneous transfer of resorcinol and/or its  
247 metabolites of about 50% of the administered dose after 24 h, 4.8% of which being parent resorcinol (Géniès  
248 et al., 2019; Hewitt et al., 2020).

249 Although a case can be made that all the conditions might have been met to favor internal exposure in the  
250 medical cases, too many uncertainties remain regarding cutaneous absorption and metabolization of  
251 resorcinol to unambiguously validate this hypothesis. Furthermore, of note, the therapeutic use of resorcinol  
252 on damaged skin continues to be prescribed — particularly for the treatment of hidradenitis suppurativa  
253 (Docampo-Simón et al., 2022) — at concentrations of 15%.

254 The paucity of TK data precludes the evaluation of the significance of the mode of administration as a  
255 determining factor of the differences in thyroid changes between humans and rodents. In conclusion, the  
256 relevance of the effects observed in human cases under specific circumstances cannot be excluded for other

257 exposure conditions. Regardless of the interpretation of the few available TK studies, thyroid effects have  
258 nonetheless been observed in rodents — although not as severe as in human patients — and, in some  
259 instances, for much lower doses and likely lower bioavailability than on damaged skin. Therefore, the  
260 singularity of human exposure is not in itself enough to consider that TDC effects of resorcinol are not of  
261 concern.

#### 262 Rat vs. human sensitivity to thyroid disruption

263 The MoA of resorcinol on thyroid function clearly relies on inhibition of TPO activity. Ultimately, long-term  
264 TPO inhibition, as for high prolonged exposure to resorcinol, can lead to hypothyroidism and goiter formation  
265 in humans, as shown in Hashimoto thyroiditis (Caturegli et al., 2014) and in a condition caused by a TPO  
266 mutation resulting in moderate residual TPO activity resulting in the apparition of goiter only after more  
267 than 8 years of age (Narumi et al., 2017). The TPO-targeting MoA of resorcinol has been demonstrated in  
268 several *in vitro* studies using purified porcine thyroid TPO, human thyroid cell lines, rat thyroid microsomal  
269 fractions, or various cell lines transfected with human TPO gene and with different substrates (Cooksey et al.  
270 1985; Dong et al. 2020; Lindsay et al. 1992; Paul et al. 2014; Paul Friedman et al. 2016). All but one study  
271 (Paul et al., 2014) reported very high potency (low half maximal inhibitory concentration,  $IC_{50}$ ) of resorcinol  
272 to inhibit TPO. In three studies, resorcinol potency was much higher than that of the reference TPO inhibitors  
273 methylmercaptoimidazole and propylthiouracil (Cooksey et al., 1985; Dong et al., 2020; Lindsay et al., 1992).  
274 Rodents are usually considered more sensitive to thyroid disruption than humans due to lower thyroglobulin  
275 stocks, higher responsiveness to TSH, and/or lack of thyroxine binding globulin (TBG) expression in adult  
276 animals (Bartsch et al., 2018). It is therefore likely that the apparent higher sensitivity of humans to resorcinol  
277 is related to different pharmacodynamic properties of TPO. Unfortunately, no reports of interspecies  
278 differences in TPO pharmacodynamic parameters are currently available in comparable experimental  
279 designs. Further studies are therefore needed to determine whether interspecies differences in TPO  
280 sensitivity to resorcinol can contribute to the difference between rodents and humans.

281 Human relevance of the rat model is still a matter of debate. To address this issue, a workshop on thyroid  
282 disruption was held in 2017 with support from the European Commission and ANSES (European Commission,  
283 2017). Some of the conclusions of this workshop were included in the ECHA/EFSA revision of the guidance  
284 for the identification of endocrine disruptors (ECHA/EFSA et al., 2018). This guidance stated that “in the  
285 absence of substance-specific data which provide proof of the contrary, humans and rodents are considered  
286 to be equally sensitive to thyroid-disruption.” The resorcinol database illustrates the fact that rodents should  
287 not be considered by default as more sensitive than humans.

288 Despite a limited number of published cases, human clinical studies unambiguously show that resorcinol can  
289 dramatically alter thyroid function. In contrast, there is a complete lack of good quality epidemiological

290 studies to conclude on an effect of resorcinol in the context of an exposure pattern more likely to occur in  
291 the general population (lower dose, oral route, etc.). Given the effects of resorcinol on TPO, subclinical  
292 hypothyroidism can be expected. These types of thyroid changes are generally largely underdiagnosed  
293 (Andersen, 2019) and may have long-lasting deleterious effects on health, in particular during critical periods  
294 of development.

295 Are thyroid-related adverse effects properly documented in animals?

296 The only potentially thyroid-related adverse effect of resorcinol that have been investigated in animal  
297 experimental studies involve neurodevelopmental outcomes (Unpublished Study report, 2003). In rats, after  
298 developmental exposure through drinking water (0-10-40-120-360 mg/l *i.e.* mean doses of  
299 0-0.8-3.9-13.1-36.9 mg/kg/d in males and 0-0.8-5.1-15.60and 46.6 mg/kg/d in females) from 28 days prior to  
300 mating until weaning at postnatal day (PND) 21, no signs of altered neurodevelopment are demonstrated in  
301 juveniles (Unpublished Study report, 2003). The only signs of altered neurodevelopment have been noted at  
302 PND60 in F1 males and characterized by increased locomotor activity from 40 mg/L of water, *i.e.* about 4  
303 mg/d/kg bw. This could not be demonstrated in females likely due to an improper schedule of testing ignoring  
304 the stage of the estrous cycle. Estrogens are indeed well-known regulators of locomotor activity (Rosenfeld,  
305 2017; Scimonelli et al., 1999). Similarly, effects of resorcinol exposure could not be demonstrated on juvenile  
306 or adult F1 rats based on the acoustic startle response or spatial memory tests. It was concluded from these  
307 behavioral tests and from brain histology that resorcinol has no effects on the brain. However, these  
308 neurodevelopmental investigations clearly suffer from at least two major limitations. First, although the  
309 behaviors analyzed in these studies are regulated by different neural structures controlling different cellular  
310 processes and signaling pathways, the observations of the brain were global and not detailed according to  
311 their structures and functions. Second, the results obtained for spatial memory should have been corrected  
312 for increased motor activity, which can compensate for a slight memory deficit (and thus influence  
313 performance in the Biel maze swimming test). Finally, although in line with neurodevelopmental parameters  
314 that can be required in guideline studies, the histological analyses were not sufficient or appropriate for the  
315 detection of any cellular or molecular changes induced by resorcinol exposure and potential underlying TH-  
316 regulated mechanisms.

317

318 The current design of rodent studies are clearly not sufficiently sensitive to detect adverse  
319 neurodevelopmental effects related to maternal and perinatal hypothyroxinemia, as previously reported  
320 (Ramhøj et al., 2020). In this context, approaches such as AOPs (OECD, 2018, AOP 42  
321 (<https://aopwiki.org/aops/42>, AOP54 <https://aopawiki.org/aops/54>) can help in the process of hazard  
322 assessment. Two recently developed AOPs addressing the impact of decreased T4 on brain development  
323 have been published. AOP 42 reports the inhibition of TPO, which is the target of resorcinol, and subsequent

324 adverse neurodevelopmental outcomes in mammals (Crofton et al., 2019). AOP 54 describes the inhibition  
325 of Na<sup>+</sup>/I<sup>-</sup> symporter (NIS) leading to learning and memory impairment (Rolaki et al., 2019). Through different  
326 molecular initiating events, both AOPs lead to a decrease in T4 levels with neurodevelopmental  
327 consequences and establish a relationship between a decrease in T4 serum concentrations and a decrease in  
328 cognitive function, with a high weight of evidence and with high relevance during brain development (fetal  
329 and perinatal stages).

330

### 331 Conclusions

332 In conclusion, available human data (even though obtained in disease conditions and concomitantly to  
333 pharmaceutical use) unambiguously show that resorcinol can act as a TDC. These observations are consistent  
334 with *in vitro* data showing the high potency of resorcinol to inhibit TPO activity. Rodent data, although  
335 showing some degree of thyroid disruption and related adverse effects (particularly in the context of  
336 developmental exposure), are not fully consistent with human clinical and *in vitro* mechanistic data showing  
337 clear thyroid disrupting properties of resorcinol. In addition, these experimental animal studies are too  
338 limited and contain too many methodological deficiencies to be fully conclusive on their own on TDC  
339 properties of resorcinol. However, the comprehensive dataset is highly consistent in terms of biology (Figure  
340 1, Table2). Many criticisms have been raised regarding the potential impact of the routes and modes of  
341 administration. Our analysis shows that neither the route of exposure in the human cases, nor the near  
342 absence of a thyroid effect with gavage in rodents are in themselves sufficient to reject the possibility that  
343 resorcinol acts as a TDC. In particular, TK data can predict internal exposure from the different routes, but no  
344 TK studies have been conducted.

345 Our analysis examined considerations relative to interspecies differences in the regulation of thyroid  
346 function. In contrast to the general assumption that rodents are more sensitive to TDC than humans, the set  
347 of available data suggests the opposite. This highlights the need to weigh in other considerations e.g.  
348 pharmacodynamic difference, rather than just interspecies sensitivity comparison. Although TPO-dependent  
349 iodine organification is one of the most highly conserved events of TH biosynthesis among vertebrates,  
350 rodents may be nonetheless less sensitive to the inhibition of TPO than humans. Given the high level of  
351 uncertainty on this aspect, human data should not be dismissed on the basis of findings in rodents.

352 Clinical hypothyroidism and associated symptoms are reversible in adults. Accordingly, the reversibility of  
353 these effects when resorcinol treatment is interrupted can be taken as tangible proof of the cause-effect  
354 relationship between resorcinol exposure and adverse effects on health (goiter, mood disorders and  
355 tiredness) related to thyroid disruption. Within the regulatory arena, this reversibility can lead to questioning

356 whether such effects are indeed adverse. In the case of resorcinol, the effects reported in human case studies  
357 were sufficiently serious and invalidating to be considered as fully adverse, even though they are reversible.  
358 In addition, even transient, developmental thyroid disruption can induce long-term changes in neural  
359 functions and behavior. Therefore, although thyroid markers can return to normal after exposure in adults,  
360 some adverse effects may not be reversible in the context of developmental exposure.

361 Finally, although developmental exposure showed some adverse neural effects on offspring that may be  
362 related to thyroid disruption, the data are too scarce to conclude on a link between these two processes. In  
363 addition, there are no available epidemiological data in humans that investigate this hypothesis. In-depth  
364 neurobehavioral investigations in rodents and a better consideration of other biomarkers of developmental  
365 thyroid disruption could help reach an undisputed conclusion.

366 Our analysis showed that, based on human data, resorcinol can fulfill the WHO definition for ED (table2),  
367 even in the presence of discrepancies with animal experimental data. It is noteworthy that in the case of  
368 resorcinol, animal studies were limited and that more often the experimental procedures were not in nature  
369 of bringing clear conclusions on TD effects and possibly related adverse effects in particular on  
370 neurodevelopmental toxicity. Nevertheless, they have been used to challenge human data. This also  
371 illustrates that the available regulatory toxicological data/guidelines clearly need in depth improvements to  
372 accurately assess thyroid disruption. Such limits are a matter of complexity for regulatory toxicological  
373 evaluation that can lead to opposite opinions among regulatory bodies, as was the case for resorcinol in the  
374 EU: ANSES's proposal to identify resorcinol as a "substance of very high concern" due to its TD properties  
375 was not adopted by EU instances two years after its submission (ANSES, 2020; ECHA, 2020). Finally, our  
376 analysis raises the questions of how many chemicals for which human data do not exist have been or will be  
377 erroneously considered as non TDCs based solely on the perfunctory evaluation of the thyroid function  
378 required in the regulatory framework for EDC evaluation.

379

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385

386

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534 **Table 1.** Summary of the regulatory tests designed by the Organization for Economic Co-  
535 operation and development (OECD) and the American Environmental Protection Agency (US  
536 EPA) that include endpoints responsive to and/or diagnostic of thyroid disruption.

537

Test	OECD level GL reference	US EPA Tier GL reference	Last updated (OECD TG/OPPTS)	Endpoints for thyroid-related activity (from OECD TG 150)*
Hershberger Bioassay in Rats	Level 3 TG 441	Tier 1 OPPTS 890.1400	September 2009 / October 2009	Optional: <ul style="list-style-type: none"> <li>Possible liver weight increase (in combination with other thyroid-related endpoints)</li> <li>Reduction in serum T4 and T3 (anti-thyroid)</li> </ul>
Amphibian Metamorphosis Assay	Level 3 TG 231	Tier 1 OPPTS 890.1100	Septembre 2009 / October 2009	<ul style="list-style-type: none"> <li>Developmental stage</li> <li>Hind limb length</li> <li>Snout-vent length</li> <li>Thyroid gland histopathology</li> <li>Time to metamorphosis</li> </ul>
XETA: Xenopus Embryonic Thyroid Signalling Assay	Level 4 TG 248	- -	June 2019	<ul style="list-style-type: none"> <li>Increase or decrease in GFP, depending on precise mode of thyroid activity</li> </ul>
Repeated Dose 28-Day Oral Toxicity Study in Rodents	Level 4 TG 407	- OPPTS 870.3050	October 2008 / July 2000	<ul style="list-style-type: none"> <li>Possible liver weight increase (in combination with other thyroid-related endpoints)</li> <li>Histopathologic changes in thyroid (follicular cell height increase and colloid area decrease)</li> <li>Other endpoints: serum T3 and T4 decreased, TSH increased; increased thyroid weight (anti-thyroid)</li> </ul>
Repeated Dose 90-Day Oral Toxicity	Level 4 TG 408	- OPPTS 870.3100	June 2018 / August 1998	<ul style="list-style-type: none"> <li>Possible liver weight increase (in combination with other thyroid-related endpoints)</li> <li>Serum T4, T3 decreased, TSH increased</li> <li>Histopathologic changes in thyroid gland</li> <li>Changes to HDL/LDL ratio (in combination with other thyroid-related endpoints)</li> </ul>
Combined Chronic Toxicity/Carcinogenicity Studies	Level 4 TG 451-3	- OPPTS 870.4300	September 2018 / August 1998	<ul style="list-style-type: none"> <li>Increased thyroid weight.</li> <li>Possible liver weight increase (in combination with other thyroid-related endpoints)</li> <li>Histopathologic changes in thyroid gland</li> </ul>
Combined 28-Day Reproductive Screening Tests	Level 4 TG 421 TG 422	- OPPTS 870.3550 OPPTS 870.3550	July 2016 / July 2020 March 1996 / July 2020	<ul style="list-style-type: none"> <li>Increased thyroid weight.</li> <li>Histopathologic changes in thyroid gland</li> <li>Serum T4, decreased, TSH increased</li> </ul>
Prenatal Developmental Toxicity Study	Level 4 TG 414	- OPPTS 870.3700	June 2018 / August 1998	<ul style="list-style-type: none"> <li>Increased thyroid weight</li> <li>Histopathologic changes in thyroid gland</li> <li>Serum T4, decreased, TSH increased in dams</li> </ul>
Developmental Neurotoxicity Study	Level 4 TG 426	- OPPTS 870.6300	October 2007 / August 1998	Nil referenced as specific to thyroid in TG 150
Subchronic Dermal Toxicity: 90-Day Study	Level 4 TG 411	- OPPTS 870.3250	May 1981 / August 1998	<ul style="list-style-type: none"> <li>Histopathologic changes in thyroid gland</li> </ul>
28-Day (Subacute) Inhalation Toxicity Study	Level 4 TG 412	- -	June 2018	<ul style="list-style-type: none"> <li>Increased thyroid weight</li> <li>Histopathologic changes in thyroid gland</li> </ul>
Subchronic Inhalation Toxicity: 90-Day Study	Level 4 TG 413	- OPPTS 870.3465	June 2018 / August 1998	<ul style="list-style-type: none"> <li>Increased thyroid weight</li> <li>Histopathologic changes in thyroid gland</li> </ul>
Repeated Dose 90-Day Oral Toxicity Study in Non-Rodents	Level 4 TG 409	- OPPTS 870.3150	September 1998 / August 1998	<ul style="list-style-type: none"> <li>Increased thyroid weight</li> <li>Histopathologic changes in thyroid gland</li> </ul>
Pubertal Development and Thyroid Function Assay in Peripubertal Male Rats	Level 4	Tier 1 OPPTS 890.1500	October 2009	<ul style="list-style-type: none"> <li>Increased thyroid weight</li> <li>Possible liver weight increase (in combination with other thyroid-related endpoints)</li> <li>Histopathologic changes in thyroid (follicular cell height increase and colloid area decrease)</li> <li>Serum T4 decreased, TSH increased</li> </ul>
Pubertal Development and Thyroid Function Assay in Peripubertal female Rats	Level 4	Tier 1 OPPTS 890.1450	October 2009	<ul style="list-style-type: none"> <li>Increased thyroid weight</li> <li>Possible liver weight increase (in combination with other thyroid-related endpoints)</li> <li>Histopathologic changes in thyroid (follicular cell height increase and colloid area decrease)</li> <li>Serum T4 decreased, TSH increased</li> </ul>
Larval Amphibian Growth and Development Assay	Level 4 TG 241	Tier 2 OPPTS 890.2300	July 2015 / July 2015	<ul style="list-style-type: none"> <li>Thyroid histopathology</li> <li>Time to metamorphosis</li> </ul>
Two-Generation Reproduction Toxicity Study	Level 5 TG 416	- OPPTS 870.3800	January 2001 / August 1998	<ul style="list-style-type: none"> <li>Increased thyroid weight</li> <li>Possible liver weight increase (in combination with other thyroid-related endpoints)</li> <li>Histopathologic changes in thyroid (follicular cell height increase and colloid area decrease)</li> </ul>
Extended One-Generation Reproductive Toxicity Study	Level 5 TG 443	- -	June 2018	<ul style="list-style-type: none"> <li>Increased thyroid weight</li> <li>Possible liver weight increase (in combination with other thyroid-related endpoints)</li> <li>Histopathologic changes in thyroid</li> <li>Serum T4, decreased, TSH increased</li> </ul>
ATGT: Avian Two-Generation Toxicity Test in the Japanese Quail	Level 5	Tier 2 OCSP 890.2100	July 2015	<ul style="list-style-type: none"> <li>T3, T4</li> </ul>

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543 **Table2 : summary of the main lines of evidence and biological plausibility for resorcinol to fulfill**  
 544 **the criteria of the definition of an endocrine disruptor as recommended by JRC**

545 Details of the reference articles are given in the supplementary material

546

Adverse effects	Plausible ED MoA/ key events:	Human relevance
<b>Goitre and severe hypothyroidism in humans</b> Bull & Fraser 1950; Hobson 1951; Berthezene 1973 ;Katin 1977	<b>Molecular initiating event= Inhibition of TPO</b> (key enzyme of TH synthesis) Taurog 1970; Lindsay 1992; Paul 2014; Jomaa 2015; Divi & Doerge 1994; Paul Friedman 2016.	<b>Effects and MoA identified in humans</b>  <i>in vitro</i> data showing TPO inhibition in human cell line
<b>Decreased T4 in humans</b> Berthezene 1973; Katin 1977	<b>Decreased iodine organification in vitro</b> Fawcett & Kirkwood 1953; Lindsay 1992; Coval & Taurog 1970.	ECHA/EFSA (2018) : humans and rodents considered equally sensitive to thyroid-disruption
<b>Decreased T4 in rodents with exposure with diet or drinking water.</b> Cooksey 1985; Main two-generation study (Unpub. 2005a); Berthezene 1979	<b>Decreased iodine organification in vivo</b> Cooksey 1985; Berthezene 1979	
<b>Histological thyroid alterations and/or increased thyroid weight in experimental animals</b> Doniach 1953; Seffner 1995; Main two-generation study (Unpub. 2005a); Cheymol 1951; Samuel 1955; Cooksey 1985; Berthezene 1979.	<b>Biological plausibility of a causal link</b> highly supported by current knowledge on neurodevelopmental effects (AOP 42)	
<b>Histological thyroid alterations and/or increased thyroid weight in human</b> Bull & Fraser 1950		
<b>Neural effects</b> Indications of motor dysfunctions (low level of evidence): Increased landing footsplay in 90-day study (Unpub. 2004) and increased motor activity in the preliminary two-generation study (Unpub. 2003).		

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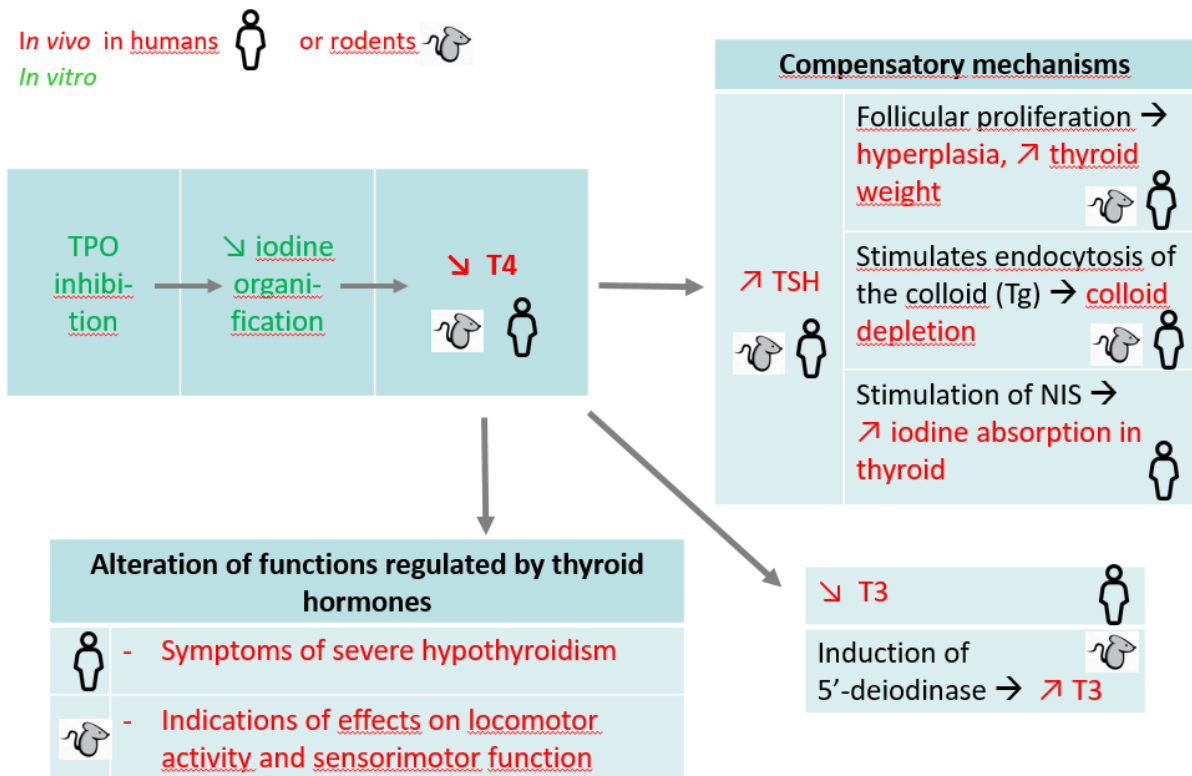
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550 **Figure caption**

551 **Figure 1.** Plausible biological links between the different thyroid-related alterations observed after exposure  
552 to resorcinol.

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