COSMETICS EUROPE CONTRIBUTION TO EU STAKEHOLDER CONSULTATION ON THE "DRAFT PRIORITY LIST OF POTENTIAL ENDOCRINE DISRUPTORS"

Part II: Ingredient Evaluation with regard to human health BPR/PPPR Criteria

RESORCINOL

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Resorcinol: CE EVALUATION WITH REGARD TO HUMAN HEALTH BPR & PPPR CRITERIA

INCI Name: **Resorcinol**

Chemical Names: **Resorcinol; 1,3-benzenediol,**

CAS RN: **108-46-3**

EXECUTIVE SUMMARY

- There is *in vitro* evidence of anti-thyroid activity of Resorcinol, specifically through TPO (Thyroid Peroxidase) inhibition (OECD conceptual framework "Level 2" evidence).
- Some rat studies with specific exposure conditions consisting of slow and/or continuous release of Resorcinol showed some anti-thyroid activity of Resorcinol that are likely related to the above activity (OECD conceptual framework "Level 3" evidence). However, the specific exposure conditions needed to produce thyroid effects in rats as well as the rat-specific susceptibility to thyroid toxicity overall cast serious doubt on the relevance of such effects to Humans and human exposure conditions.
- No adverse effects that may be mediated by an endocrine mode of action (including anti-thyroid activity) were observed in pivotal toxicity studies on Resorcinol (OECD 408, 414 & 416 studies, *i.e.* absence of OECD conceptual framework "Level 4" or "Level 5" evidence).
- Historical cases reporting thyroid changes in Humans exposed among others to large doses of Resorcinol applied onto compromised or absent skin over long treatment periods are available. However, since these individuals were under several other medications, the relationship of the effects observed to Resorcinol exposure remains doubtful. Additionally, the reversibility of the effects observed as well as the very specific exposure conditions used decrease the weight of the associated evidence in the context of the present evaluation.
- Altogether, the evidence available is considered to be insufficient for Resorcinol to meet BPR (Biocidal Products Regulation) & PPPR (Plant Protection Products Regulation) criteria for identifying Endocrine Disrupting Chemicals (EDCs).
- These views are in line with the conclusions drawn by tukes (the Finnish Safety and Chemicals Agency), *i.e.* the Member State Competent Authority in charge of the evaluation of Resorcinol in the context of the REACh regulation.

BACKGROUND

Positive opinions have been granted by several EU regulatory agencies and scientific committees following their evaluation of Resorcinol -*i.e.* by tukes (the Finnish Competent Authority for REACh) (1), the European Food Safety Authority (2) and the EU Scientific Committee for Consumer Safety (3)-. However, the safety of this substance is still questioned due to an alleged potential for endocrine disruption (ED) targeting the Thyroid gland.

These concerns have their origins in historical cases reporting thyroid changes in Humans exposed among others to large doses of Resorcinol applied onto compromised or absent skin over long treatment periods, in spite of the absence of recent evidence of such effects in animals and in Humans.

Finland first listed Resorcinol on the CoRAP (Community Rolling Action Plan) of EChA (European Chemicals Agency) in 2013 for suspected ED properties. The Finnish Safety and Chemicals Agency (tukes) closed its substance evaluation in October 2017 without asking for additional information and went on a RMOA (Risk Management Options Analysis). The conclusions of the RMOA published in 2018 confirmed that Resorcinol is not eligible as SVHC (Substance of very high concern) or for REACh restrictions and that other risk management options, *i.e.* classification and labelling, are more appropriate measures to address the potential safety concerns of this substance.

In this context, the following hazard and mode of action evaluation aims at evaluating whether Resorcinol meets the EDC definition criteria recently accepted in the context of the Biocidal Products Regulation (BPR) & the Plant Protection Products Regulation (PPPR) (4, 5).

TABULATED SUMMARY OF THE EVIDENCE AVAILABLE

The table below summarizes the scientifically relevant information (*i.e.* taken from studies rated 1 or 2 according to the so-called "Klimisch code" (6)) evaluated in the present document, classified according to the OECD "Conceptual Framework for testing and assessment of endocrine disrupters" (7).

Only the information related to anti-thyroid activity & thyroid toxicity was included, as this is the only endpoint of endocrine controversy for Resorcinol.

OECD Conceptual Framework Level	Studies Available	Results
Level 1 *Existing Data and Non-Test Information*		
Level 2 *In vitro assays providing data about selected endocrine mechanism(s) / pathways(s)*	*In vitro* exploratory study	In porcine thyroid gland slices, Resorcinol concentrations of 2×10^{-7} to 5×10^{-5} mol/L caused a significant decrease of ^{125}I uptake and its incorporation into tyrosine to form iodotyrosines. A Resorcinol concentration of 0.3 µmol/L caused a 50% inhibition of thyroid peroxidase (8).
Level 3 *In vivo* assays providing data about selected endocrine *mechanism(s) / pathways(s)*		Effects on the thyroid gland (increase of the mean epithelial cell height of follicular epithelial cells and increased formation of new follicles) in rats after daily oral administration of 0.004 % Resorcinol in drinking water over 12 weeks (9)

OECD Conceptual Framework Level	Studies Available	Results
Level 4 *In vivo assays providing data on adverse effects on endocrine-relevant endpoints*	Repeat-dose 90-day study (OECD 408)	Rat, oral (gavage), 0, 40, 80, 250 mg/kg bw, 13 weeks (93 days), NOAEL 80 mg/kg bw, at 250 mg/kg bw/day clinical signs and mortality, no significant changes in thyroid weights, and no relevant histopathological abnormalities) (10)
	Prenatal developmental toxicity (OECD 414)	Rat, oral (gavage), 0, 40, 80, 250 mg/kg bw, once daily, GD 6-19, no adverse developmental effects, maternal NOAEL 80 mg/kg bw, developmental NOAEL 250 mg/kg bw (10)
Level 5 *In vivo assays providing more comprehensive data on adverse effects on endocrine-relevant endpoints over more extensive parts of the life cycle of the organism*	Mammalian Two-Generation Study (OECD 416)	Rat, oral (drinking water), 0, 120, 360, 1000, 3000 mg/L, no adverse effects on either reproductive or thyroid gland end points, *i.e.* no statistically significant test article-related changes in the mean concentrations of T3, T4 or TSH were noted in the F0 or F1 parental animals or in the F1 or F2 pups (PND 4 or PND 21). The NOAEL was 3000 mg/L, which corresponds to ~233 mg/kg bw/day for males over the entire generation, 304 mg/kg bw/day for females during premating and gestation periods and 660 mg/kg bw/day for females during lactation period (11)

EVIDENCE OF ENDOCRINE-RELATED BIOLOGICAL ACTIVITY OF RESORCINOL

Mechanistic evidence of endocrine activity/mode of action:
Resorcinol can inhibit Thyroid Peroxidase (TPO) *in vitro* (IC50 of of 0.3 µM), and in porcine thyroid gland slices, Resorcinol concentrations of 2×10^{-7} to 5×10^{-5} M caused a significant decrease of ^{125}I uptake and its incorporation into tyrosine to form iodotyrosines (8).
However, exposure of rats to Resorcinol without any specific interference with its rapid metabolism and clearance (e.g. through subcutaneous injections in oily solution or through other means ensuring continuous administration) caused no reduction of blood thyroid hormone levels and did not result in either thyroid dysfunction or developmental neurotoxicity.
Some effects on the thyroid gland were reported in animals only under particular exposure conditions that are not clinically relevant. Finally, the evidence available clearly shows that when compared to humans, rats are more susceptible to TPO inhibition and are more susceptible to thyroid toxicity in general, due to the lack of high-affinity thyroxin-binding globulin in these species (12,13).

EVIDENCE OF ADVERSE EFFECTS OF RESORCINOL IN INTACT ORGANISMS OR THEIR PROGENY

Summary of pivotal toxicity studies (OECD Conceptual Framework "Level 4 & "Level 5" studies)

Summary of OECD 408 study

- Rats were given 0, 40, 80, 250 mg/kg bw Resorcinol once a day by oral gavage for at least 13 weeks (93 days) in a GLP-compliant study that used degassed purified water as vehicle. At 250 mg/kg bw/day, all males and females (including satellites) showed intermittent convulsive movements, starting between weeks 6 and 8 and lasting until the end of the treatment period. Also excessive salivation (majority of animals) and loud breathing (2 males) was reported in the 250 mg/kg bw/day group. Mortality was mentioned in the 80 mg/kg bw/day (2 males) and

the 250 mg/kg bw/day dosage group (1 female). According to the study report, observed deaths at these dose levels were not treatment-related but may be caused by lung lesions due to incidental gavage errors. With the exception of the two males which had convulsions and died, no clinical observations were recorded at 80 mg/kg bw/day. No treatment-related effects on body weight, food consumption, blood and urine parameters, organ weights and necropsy findings were noted. The female group receiving 250 mg/kg bw/day gained slightly less weight (86% of the weight gained by the controls). Examination of the animals during the Functional Observation Battery did not reveal any treatment-related effect. Under the experimental conditions of the study, the NOEL was reported to be 80 mg/kg bw/day.

Summary of OECD 414 study

- Rats were given 0, 40, 80, 250 mg/kg bw Resorcinol once a day by oral gavage throughout the gestation period (GD6-19) in a GLP-compliant study. At 250 mg/kg bw/day the net body weight change was significantly reduced. No other maternal effects were observed. All group mean numbers of implantations and live fetuses and the extent of pre- and post-implantation losses were comparable with the controls. There were no effects of treatment on foetal body weight. In the litters, no external, soft tissue or skeletal malformations or variations were considered to be treatment-related. There was a significantly increase in the incidence of foetuses with an incompletely ossified interparietal at 40 and 80 mg/kg bw/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 bw/day, when compared to controls ($p<0.05$). In the absence of any effects at 250 mg/kg bw/day these observations were not considered to be treatment-related. The maternal NOAEL of Resorcinol administered by gavage to pregnant female rats was 80 mg/kg bw/day and the developmental NOAEL was 250 mg/kg bw/day.

Summary of OECD 416 study

- Rats were given Resorcinol by the oral route in the drinking water for at least 70 consecutive days in a GLP-compliant study (drinking water concentrations of 0, 120, 360, 1000 and 3000 mg/L for the F0 and F1 generations). No F0 or F1 parental test article-related deaths or clinical findings were reported. Mean body weights and body weight gains were affected in the 3000 mg/L treatment group F0 and F1 animals. The mean water consumption was decreased with ~10% in the 1000 mg/L (F0 animals only) and with ~20% in the 3000 mg/L treatment group (F0 and F1 animals) due to the poor palatability of high concentration of water containing Resorcinol. Decreases in water consumption were not associated with effects on food intake and food utilization. Reproductive performance (oestrous cycles, mating and fertility indices, number of days between pairing and coitus, and gestation length) and parturition in the F0 and F1 animals were unaffected by the test article. Spermatogenic endpoints (mean testicular and epididymal sperm numbers and sperm production rate, motility, progressive motility and morphology) in the F0 and F1 males were unaffected by the test article. No test article-related effects were observed on F1 and F2 pup survival or the general physical condition of the pups during the pre-weaning period. No test article-related macroscopic findings, organ weight or adverse microscopic target-organ effects were observed in the F0 or F1 parental animals. In addition, no test article-related macroscopic findings or effects on organ weights were noted in the F1 or F2 pups at the scheduled necropsies; no test article-related macroscopic findings were noted for found dead F1 or F2 pups. No statistically significant test article-related changes in the mean concentrations of T3, T4 or TSH were noted in the F0 or F1 parental animals or in the F1 or F2 pups selected for analysis (PND 4 or PND 21). The higher (but non-significant) TSH values noted at all dose levels in the F0 males at the scheduled necropsy were

not considered test article-related in the absence of effects on T3 or T4, organ weights or adverse macroscopic or microscopic findings. Test article-related decreased colloid within the thyroid glands of the 3000 mg/L F0 males was not considered adverse due to the lack of associated functional effects. Based on the results of this study, the NOAEL was considered to be 3000 mg Resorcinol/L, which corresponds to ~233 mg/kg bw/day for males over the entire generation, 304 mg/kg bw/day for females during premating and gestation and 660 mg/kg bw/day for females during lactation.

Summary of potentially endocrine-mediated adverse effects observed in pivotal toxicity studies

In the above pivotal toxicity studies on Resorcinol (OECD 408, 414 & 416 studies), there were no adverse effects that may be mediated by an endocrine mode of action. Specifically, there was no evidence of thyroid toxicity, thyroid dysfunctions, malformations or consequences thereof.

Additional evidence from animal studies

Evidence of anti-thyroid activity of Resorcinol in animals was only observed under conditions of continuous exposure to high doses (154 mg/kg bw/d by the sub-cutaneous (SC) route, ≥47 d; 3000 mg/kg bw/d via diet, 2 weeks; 750 mg/kg bw/d dermal, 4 weeks), and required a hydrophobic vehicle for the SC injection such as arachis oil or peanut oil in order to establish a reservoir of Resorcinol so that Resorcinol was continuously bioavailable (11,14,15,16).

Additional evidence from observations in Humans

Isolated case reports from over 50 years ago showing reversible thyroid changes have not been substantiated since that period in either consumers or industrial workers under current exposure conditions. These case reports involved individuals under several medications, and effects were reported after topical application over several months of ointments containing high doses of Resorcinol, under conditions of compromised or absent skin. Extended exposure to high levels of free Resorcinol, when applied to open wounds, led to several medical case reports reporting goiters (17, 18, 19, 20). As with animal studies, these cases reporting effects on the thyroid gland in humans are considered to be related to continued exposures at very high dose levels over long treatment periods.

No thyroid effects were observed in a clinical study after daily topical exposure to a formulation with 2% Resorcinol over a period of 4 weeks. Resorcinol was applied topically to the face, shoulders, upper chest and upper back of 3 healthy men, twice a day, and 6 days/week over a period of 4 weeks. Resorcinol was applied at 2% in a 20 ml hydro-alcoholic vehicle (800 mg Resorcinol per day corresponding to a daily dose 12 mg/kg bw and 0.30 mg/cm2). This is equivalent to an estimated systemic dose level of 0.4 mg/kg bw/day. No detectable levels of free Resorcinol or its conjugates were found in blood (detection limit of the method applied was 0.1 µg/ml). In 24-hour urine samples collected after 14 days of continuous treatment, a maximum of 0.47 to 2.87% (up to 23 mg Resorcinol) of the applied daily dose was excreted and detected as the glucuronide and sulphate conjugates. Blood chemistry and thyroid function (T3, T4, T7 and TSH) were normal throughout the study (21).

PLAUSIBILITY OF RESORCINOL-INDUCED ADVERSE EFFECTS BEING THE CONSEQUENCE OF AN ENDOCRINE MODE OF ACTION

- Animal pivotal toxicity studies: Not Applicable, given that no potentially endocrine-related effects were observed in animal pivotal toxicity studies on Resorcinol.
- Additional evidence from animal studies: the "anti-thyroid" mode of action of Resorcinol reported above is likely responsible for the effects reported in rats dosed continuously with Resorcinol.
- Additional evidence from observations in Humans: the relationship of the thyroid changes observed in the above case reports with the exposure to Resorcinol is doubtful, specifically because these individuals were under several other medications than Resorcinol.

DISCUSSION - CONCLUSION

Though the "anti-thyroid" mode of action of Resorcinol through TPO inhibition is likely responsible for the effects reported in rats dosed continuously with Resorcinol, these effects are considered not to be sufficient for Resorcinol to meet the BPR & PPPR criteria for identifying EDCs.
This view is mainly because such effects can only be observed under specific conditions of slow/continuous release: as confirmed by EFSA in their scientific opinion on the use of Resorcinol as a food additive, the rapid metabolism and subsequent clearance of Resorcinol from the systemic circulation under normal exposure conditions indeed prevents the sustained delivery of possible toxic doses of Resorcinol to the thyroid gland for effects to occur (3,16), which is consistent with the absence of any effects on the thyroid in pivotal toxicity studies.
Additionally, it is generally recognized that any effects on the thyroid gland observed in rats should be interpreted with caution: long-term alterations of the pituitary-thyroid axis are more likely to predispose a higher rate of proliferative lesions such as hyperplasia in rats than in humans. In addition, the higher susceptibility of rats to thyroid toxicity when compared to Humans is also related to a shorter plasma half-life of T4 (about 12–24 h in rats *vs* 5–9 days in humans), due to considerable species differences in the transport proteins for thyroid hormones (in humans, the circulating T4 is bound primarily to thyroxine-binding globulin, whereas this protein is not present in rodents) (2,12,13,14,15).

Historical cases reporting evidence of reversible thyroid changes in Humans exposed over long treatment periods to large doses of Resorcinol applied onto compromised or absent skin are available. However, the evidence from these case reports is considered not to be sufficient for Resorcinol to meet the BPR & PPPR criteria for identifying EDCs. This is mainly because the causal relationship between the thyroid effects observed and exposure to Resorcinol remains highly doubtful due to the individuals involved being under several other medications than Resorcinol. Additionally, the reversibility of the effects observed as well as their occurrence in injured individuals was also taken into account.

Accordingly, Resorcinol is considered not to meet agreed scientific criteria for identifying EDCs, which conclusion is in line with a recent evaluation by Finnish tukes, the Member State Competent Authority in charge of the evaluation of Resorcinol in the context of the REACh regulation, which concluded that Resorcinol is not eligible as SVHC (Substance of very high concern) or for REACh restrictions (1).

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