EUROPEAN CHEMICALS AGENCY

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DECISION ON SUBSTANCE EVALUATION PURSUANT TO ARTICLE 46(1) OF REGULATION (EC) NO 1907/2006

For 4,4'-sulfonyldiphenol, CAS No 80-09-1 (EC No 201-250-5)

Addressees: Registrant(s)[1] of 4,4'-sulfonyldiphenol (Registrant(s))

This decision is addressed to the Registrant(s) of the above substance with active registrations pursuant to Article 6 of the REACH Regulation on the date on which the draft for the decision was first sent for comments. If Registrant(s) ceased manufacture upon receipt of the draft decision pursuant to Article 50(3) of the REACH Regulation, they did not become addressee(s) of the decision. A list of all the relevant registration numbers of the Registrant(s) that are addressees of the present decision is provided as an Annex to this decision.

Based on an evaluation by the Belgian Federal Public Service Health, Food Chain Safety and Environment, Risk Management Service as the Competent Authority of Belgium (evaluating MSCA), the European Chemicals Agency (ECHA) has taken the following decision in accordance with the procedure set out in Articles 50 and 52 of Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH Regulation).

This decision is based on the registration dossier(s) on 27 February 2015, i.e. the day until which the evaluating MSCA granted an extension for submitting dossier updates which it would take into consideration.

This decision does not imply that the information provided by the Registrant(s) in the registration(s) is in compliance with the REACH requirements. The decision neither prevents ECHA from initiating compliance checks on the dossier(s) of the Registrant(s) at a later stage, nor does it prevent a subsequent decision under the current substance evaluation or a new substance evaluation process once the present substance evaluation has been completed.

I. Procedure

Pursuant to Article 45(4) of the REACH Regulation the Competent Authority of Belgium has initiated substance evaluation for 4,4'-sulfonyldiphenol, CAS No 80-09-1 (EC No 201-250-5) based on registration(s) submitted by the Registrant(s) and other relevant and available information and prepared the present decision in accordance with Article 46(1) of the REACH Regulation.

[1] The term Registrant(s) is used throughout the decision, irrespective of the number of registrants addressed by the decision.

On the basis of an opinion of the ECHA Member State Committee and due to initial grounds for concern relating to Human health/Suspected CMR; Potential endocrine disruptor; Exposure/aggregated tonnage, 4,4'-sulfonyldiphenol (also known as Bisphenol S, BPS) was included in the Community rolling action plan (CoRAP) for substance evaluation to be evaluated in 2014. The updated CoRAP was published on the ECHA website on 26 March 2014. The Competent Authority of Belgium was appointed to carry out the evaluation.

The evaluating MSCA considered that further information was required to clarify the abovementioned concerns. Therefore, it prepared a draft decision pursuant to Article 46(1) of the REACH Regulation to request further information. It submitted the draft decision to ECHA on 19 March 2015.

The registration dossier was updated on 27 February 2015 by the Registrant(s) with the results of the OECD TG 408 and OECD TG 414 studies, following approval of testing proposals, decision number TPE-D-0000002019-79-05/F.

On 4 May 2015 ECHA sent the draft decision to the Registrant(s) and invited them pursuant to Article 50(1) of the REACH Regulation to provide comments within 30 days of the receipt of the draft decision.

Registrant(s) commenting phase

By 10 June 2015 ECHA received comments from the Registrant(s) of which it informed the evaluating MSCA without delay.

The evaluating MSCA took into account the comments received from the Registrant(s) and on basis of this information, section II (Information required) was amended: a request for a Fish Lifecycle Toxicity Test (FLCTT) was added. Section III (Statement of Reasons) was modified accordingly, to include the reasons for the FLCTT and to cover other comments received.

Commenting by other MSCAs and ECHA

In accordance with Article 52(1) of the REACH Regulation, on 21 January 2016 the evaluating MSCA notified the Competent Authorities of the other Member States and ECHA of its draft decision and invited them pursuant to Articles 52(2) and 51(2) of the REACH Regulation to submit proposals to amend the draft decision within 30 days of the receipt of the notification.

Subsequently, Competent Authorities of the Member States and ECHA submitted proposals for amendment to the draft decision.

On 26 February 2016 ECHA notified the Registrant(s) of the proposals for amendment to the draft decision and invited them pursuant to Articles 52(2) and 51(5) of the REACH Regulation to provide comments on those proposals for amendment within 30 days of the receipt of the notification.

The evaluating MSCA reviewed the proposals for amendment received and amended the draft decision.

Referral to Member State Committee

On 7 March 2016 ECHA referred the draft decision to the Member State Committee.

On 29 March 2016, in accordance to Article 52(2) and Article 51(5), the Registrant(s) provided comments on the proposals for amendment. The Member State Committee took the comments of the Registrant(s) on the proposal(s) for amendment into account.

After discussion in the Member State Committee meeting on 25-29 April 2016, a unanimous agreement of the Member State Committee on the draft decision as modified at the meeting was reached on 27 April 2016. ECHA took the decision pursuant to Article 52(2) and Article 51(6) of the REACH Regulation.

II. Information required

Pursuant to Article 46(1) of the REACH Regulation the Registrant(s) shall submit the following information using the indicated test methods (in accordance with Article 13 (3) and (4) of the REACH Regulation) and the registered substance subject to the present decision:

1. Extended One Generation Reproductive Toxicity Study in Sprague-Dawley rats, oral route, according to test method OECD 443, with the developmental neurotoxicity and immunotoxicity (DNT/DIT) cohorts and with the conditional extension of Cohort 1B to mate the F1 animals to produce an F2 generation, as specified in Section III.

2. Toxicokinetics (test method: EU B.36/OECD 417).

3. *In vivo* alkaline comet assay performed in rats by oral administration (gavage), (test method: OECD 489) on tissues as specified in Section III. This request is conditional to the results of the Toxicokinetics, as specified in Section III.

4. Medaka or Zebrafish Extended One Generation Reprodution test (MEOGRT or ZEORGT) including a range finding study for ED specific endpoints, as specified in section III.

5. Exposure data and exposure assessment as specified in Section III.

Furthermore, pursuant to Article 46(1) of the REACH Regulation the Registrant(s) shall submit full study reports for the information required under points 1 to 4 of this section II. Indeed a complete rational and an access to the whole available information (implemented method, raw data collected, interpretations and calculations, consideration of uncertainties, argumentation etc.) are needed to fully assess the provided information and to efficiently clarify the concerns.

Deadline for submitting the required information

Pursuant to Article 46(2) of the REACH Regulation, the Registrant(s) shall submit to ECHA by **20 September 2018** an update of the registration(s) containing the information required under point 1-4 above[2], including robust study summaries and full study reports and, where relevant, an update of the Chemical Safety Report.

[2] The deadline set by the decision already takes into account the time that registrants may require to agree on who is to perform any required tests and the time that ECHA would require to designate a registrant to carry out the test(s) in the absence of the aforementioned agreement by the registrants (Article 53(1) of the REACH Regulation).

The Registrant(s) asked for an extension of this deadline. ECHA considers that no justification is provided by the Registrant(s) and therefore maintains the proposed 27 months deadline.

Regarding information required under point 5 above, the Registrant(s) shall submit to ECHA the information by **20 September 2017.**

III. Statement of reasons

Based on the evaluation of all relevant information submitted on 4,4'-sulfonyldiphenol and other relevant and available information, ECHA concludes that further information is required in order to enable the evaluating MSCA to complete the evaluation of whether the substance constitutes a risk to human health or the environment.

1. Extended-one generation reproductive toxicity study in rats, oral route, with the developmental neurotoxicity and immunotoxicity (DNT/DIT) cohorts and with the conditional extension of Cohort 1B to mate the F1 animals to produce an F2 generation

The Concern(s) Identified

According to the IPCS/WHO (2002) definition, "An ED 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)population."This means that a chemical is identified as an ED if an adverse *in vivo* effect can be plausibly linked to an endocrine mode of action.

In vitro tests and *in vivo* studies such as those presented below show **endocrine disrupting (ED) modes of action** for 4,4'-sulfonyldiphenol. These are studies from level 2 and 3 of the "OECD conceptual framework and standardized test guidelines for evaluating chemicals for endocrine disruption" (OECD guidance document No. 150):

Several *in vitro* assays, corresponding to the **OECD Conceptual Framework (CF) level 2**, show weak agonist estrogenic activity of 4,4'-sulfonyldiphenol (ER binding affinity, ER transactivation and MCF7 proliferation)(e.g. Akahori *et al.* 2008, Kang *et al.* 2014, Molina-Molina *et al.* 2013). Other CF level 2 *in vitro* assays also show an impact on steroidogenesis (Rosenmai *et al.* 2014, Goldinger *et al. 2015*). No conclusion can be drawn regarding androgenic activity (contradictory results)(Kitamura *et al.* 2005, Teng *et al.* 2013, Molina-Molina *et al.* 2013, Rosenmai *et al.* 2014, Roelofs *et al.* 2015).

One uterotrophic assay in immature rat, which corresponds to the **OECD CF level 3** (*in vivo* study), confirmed endocrine activity of 4,4'-sulfonyldiphenol *in vivo* (Yamasaki *et al.*, 2004) (estrogenic activity and/or other modes of action via the HPG axis). The data provided in Akahori *et al.*, 2008 seem to be a reassessment of the data from Yamasaki *et al.*, 2004.

Tests from **OECD CF level 4** provide information about **adverse effects**:

- In the **Reproduction/Developmental Toxicity Screening Test** (OECD TG 421)(2000) with Sprague-Dawley rats, the evaluating MSCA identified a concern for reproduction. The female reproductive performances are clearly reduced by 4,4'-sulfonyldiphenol in the **300 mg/kg bw/day** group: 5 out of 12 female rats did not become pregnant and the 7 females with progeny have a reduced number of pups (9.1 vs 14.3 in the control, but not considered statistically significant in the study report).

There was a statistically significant prolongation of oestrous cycle and decreased implantation index. No histopathological abnormalities (in the ovaries, uterus) were observed.

Results of the **Repeated Dose 90-Day Oral Toxicity Study** in Wistar rats (OECD TG 408)(2014) and of the **Prenatal Development Toxicity Study** in Wistar rats (OECD TG 414)(2014) were assessed by the evaluating MSCA after the draft decision was issued to the Registrant(s). The registration dossier(s) were updated with both studies on 27 February 2015.

- No teratogenic effects were seen in the **Prenatal Development Toxicity study**, where dams were exposed to 4,4'-sulfonyldiphenol (30, 100 and 300 mg/kg bw/day) from gestational day 6 to 19.

- Some adverse effects on organs linked to the endocrine system were observed in the **Repeated Dose 90-Day Oral Toxicity Study**. In this study, rats were exposed to 100, 300 and 1000/600 mg/kg bw/d of 4,4'-sulfonyldiphenol (the highest dose was reduced to 600 in male animals at day 70 due to severely impaired body weight development):
  - At the highest dose, the absolute and relative weight of adrenal glands were significantly increased in males and females. This was correlated with histopathological findings (hypertrophy/hyperplasia) in males (in 8 males/10);
  - The mammary glands of male animals show atrophy (change from the physiological lobulo - alveolar morphology to a tubulo-alveolar appearance with smaller more basophilic epithelial lining cells) at mid- (in 7 animals/10) and high dose group (in 10/10); focal squamous cell metaplasia of glandular epithelium in the uterus(dose dependent increased incidence: 0/10, 2/10, 2/10, 5/10, in control, low, mid and high dose groups, respectively).

No **OECD CF level 5** test is available for 4,4'-sulfonyldiphenol.

In conclusion, taking into account the high aggregated tonnage (1000-10000 T/year) and the uses of the substance, a risk for human health cannot be excluded. Endocrine disruption properties of the substance need to be clarified.

Why new information is needed

Currently, the substance has no harmonized classification. The results of an Extended one-generation reproductive toxicity study (EOGRTS) will elucidate Human Health ED adverse effects. This could lead to an harmonized classification for reproduction and/or to an identification of the substance as Substance of very high concern (SVHC) (reprotoxic and/or ED for Human Health) and possible inclusion in Annex XIV of the REACH Regulation (for human health).

Considerations on the test method and testing strategy

The OECD 421 test is an apical assay providing data on adverse effects related to reproduction and development. This study is however not designed to detect EDs but some of the endpoints are relevant for the assessment of possible endocrine disruption (Positive results on reproductive endpoints and parental organ endpoints) (OECD GD 150).

As some adverse effects are observed in the OECD 421 test (fertility impairment at 300 mg/kg bw/day), together with in vitro mechanistic data (demonstrating oestrogenic activity) and other in vivo data (e.g. see above, results from the 90-day study), following option is proposed in the OECD guidance n°150 to increase evidence: Perform assay from level 5 (*e.g.* ext-1 or 2-gen assay) (scenario A in Table Annex 2.8).

As the OECD TG 443 (EOGRTS) has been optimized with regard to evaluation of endpoints of relevance for endocrine disruption, this method is the preferred one. Moreover, besides endocrine parameters, this method includes relevant data on reproductive toxicity, including developmental neurotoxicity and immunotoxicity.

The extended one-generation reproductive toxicity study (EOGRTS) is requested with the following study design:

i. Conditional extension of Cohort 1B to produce the F2 generation

In REACH Annex X, significant exposure (consumers and professionals) and indications of relevant mode(s) of action related to ED (from in vivo studies or non-animal approaches), are triggers for the inclusion of the extension of Cohort 1B to produce the F2 generation. Those triggers are further explained in ECHA guidance (Chapter R.7a).

Due to the growing concerns and restrictions on 4,4'-isopropylidenediphenol (Bisphenol A), the use of 4,4'-sulfonyldiphenol as alternative is growing. In 2012, Liao *et al.* found 4,4'-sulfonyldiphenol already in 81% of urine samples (Liao et al, 2012a). Recently, Ye *et al.* (2015) analyzed 616 archived urine samples from US adults collected between 2000 and 2014. They showed that the detection and the concentration of BPS are rising through years. Moreover, concerns are growing regarding occupational exposure of cashiers. 4,4'-sulfonyldiphenol has been detected in thermal paper (Liao *et al.*, 2012b, Goldinger *et al.*, 2015, Thayer *et al.*, 2016), and a recent study showed that the concentration of 4,4'-sulfonyldiphenol in cashiers double in urine after their work day in comparison with non-cashiers control group (Thayer *et al.*, 2016). There is thus concern regarding 4,4'-sulfonyldiphenol exposure of both consumers and professionals.

Available data on 4,4'-sulfonyldiphenol already show some ED concern at a screening stage. The OECD 421 test indeed shows some adverse effects on fertility after parental exposure. The extension of the Cohort 1B (mating of the Cohort 1B animals to produce the F2 generation) will provide information on the fertility of the offspring, i.e. the F1 generation, which has been exposed already during germ cell formation, preimplantation, *in utero* and postnatal periods. Due to the ED (oestrogenic) mode of action of the substance, possible impaired fertility in F1 generation could occur at lower doses than in the parental generation.

ECHA took note of the proposal for amendment (PfA) of one MSCA, namely that there is no need to produce the F2 generation. In addition, it was argued that based on the results of the OECD 421 test, not enough animals might be available to perform this extension. ECHA took note of this argument and decided that there is no need to produce the F2-generation if the effects in the parental and first generation would meet the criteria to classify as reproductive toxicant 1B according to the CLP Regulation[3].

[3] The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate (Guidance on the application of the CLP criteria).

ii. Inclusion of Cohort 2A/2B (developmental neurotoxicity)

Inclusion of the DNT cohort may be important in relation to certain types of adverse effects caused by endocrine disrupters, i.e. effects on the sexual dimorphic development of the brain.

In the ECHA guidance (Chapter R.7a: Endpoint specific guidance. Version 4.1 – October 2015)(Appendix R.7.6-2, EOGRTS Study Design), information on specific hormonal mechanisms/mode of action with clear association with the developing nervous system, such as oestrogenicity (Fryer *et al.*, 2012) is a trigger for inclusion of cohorts 2A/2B.

Hence, inclusion of this cohort is especially warranted in this case due to the oestrogenic mode of action of 4,4'-sulfonyldiphenol.

Furthermore, following effects were seen in fish after exposure to 4,4'-sulfonyldiphenol:
- effects on the hypothalamic development in Zebrafish embryos (Kinch *et al.*, 2015);
- effects on the thyroid hormones in the Naderi fish study (Naderi *et al.*, 2014).

These effects seen in fish further justify the request for the DNT Cohort.

Moreover, in aPfA, the following reference was provided: Castro *et al.* (2015), indicating possible impact of the substance on emotional responses and cognitive function. Indeed, in this study, in the prefrontal cortex (PFC) of female juvenile rats, 4,4′-sulfonyldiphenol decreased the mRNA levels of an isozyme (5α-R3) of 5α-reductase which is a rate-limiting enzyme in the biosynthesis of neurosteroids modulating emotional responses and cognitive function. Also, 4,4′-sulfonyldiphenol altered the transcription of several dopamine (DA) and serotonin (5-HT) related genes including strong induction of *Cyp2d4* implicated in corticosteroids synthesis. DA and 5-HT signalling in the PFC are implicated in high-level cognitive function. In this PfA, the Registrant(s) was advised to therefore assess the cognitive function by including learning and memory tests in the cohort 2A/2B, while paying particular attention to paragraph 50 of the OECD TG 443.

The Registrant(s) have in their comments argued that learning and memory assays do not represent a formal requirement of the DNT cohort and questioned the predictivity and sensitivity of these assays. For the Registrant(s), in order not to compromise the formal testing parameters of the DNT cohort, an additional cohort would be recommended.

In the neurobehavioral testing specification of the US National Toxicology Program (NTP) (June 10, 2015), the Morris Water Maze is proposed as the default learning and memory test. Therefore, the evaluating MSCA recommends this test for learning and memory assessment. However, if the animals appear to be compromised a priori in gait, running another learning and memory task where learning of the task would not interfere with performance due to motor effects can still be considered by the Registrant(s).

ECHA took note of the arguments of the Registrant(s), but still finds it relevant to ask for such investigation in the cohort 2A/2B, as a concern is identified based on the available mechanistic data. Moreover, this possibility for such investigation in the DNT cohort is mentioned in the OECD 443 Test guidance (paragraph 50).

Therefore, the Registrant(s) shall, in addition to conducting the DNT cohort, assess the cognitive function by including learning and memory test within the DNT cohort. The Morris Water Maze test is recommended.

iii. Inclusion of Cohort 3 (developmental immunotoxicity)

In the ECHA guidance (Chapter R.7a: Endpoint specific guidance. Version 4.1 – October 2015)(Appendix R.7.6-2, EOGRTS Study Design), information on hormonal mechanisms/modes of action with clear association with the immune system, such as oestrogenicity (Ádori *et al.*, 2010) is a trigger for inclusion of cohorts 3.

ECHA considers it important to identify the most sensitive endpoint to determine the most appropriate risk management measure (e.g. identification as SVHC).

Hence, inclusion of the DIT cohort is warranted here due to the oestrogenic mode of action of 4,4'-sulfonyldiphenol.

iv. Premating exposure duration

According to the ECHA guidance (Chapter R.7a), the pre-mating exposure duration shall be 10 weeks in order to cover the full period of spermatogenesis and folliculogenesis.

v. Dose level setting

The test guideline (OECD 443) recommends to include at least 3 dose levels and a concurrent control. Two- to four-fold intervals are optimal. The dose levels shall be based on toxic effects. The Registrant(s) should make sure that appropriate doses are selected by taking into account the available data and, if needed, performing a range finding study. ECHA strongly recommends to clarify the effect of the substance on the oestrus cycle (e.g. with vaginal smear) before start of the treatment. The doses and any effects on the oestrus cycle shall be reported in the study report.

vi. Route of exposure

The substance shall be administered via the oral route.

vii. Species

The EOGRTS shall be performed in rats.

In the comment to the PfAs, the Registrant(s) mentioned that "*As demonstrated by the recent 90-day oral toxicity study (OECD TG 408; [redacted] 2014) the substitution of the Sprague Dawley rat strain by Wistar rats confirmed the specific organ toxicity in the GI tract. However, with a lower severity and higher effect levels in Wistar rats compared to SD rats. Therefore a better differentiation of direct and indirect effects on reproduction can be expected in the proposed EOGRTS with Wistar rats.*"

In reply to this comment, ECHA clarifies that the Registrant(s) fails to demonstrate the link between reproductive effect and the caecum effects. Therefore the same rat strain used for the OECD 421 (Sprague-Dawley) shall be tested to clarify the suspected concern for reproduction and avoid any difference in sensitivity between rat strains.

Particular focus on the following histological examinations:

Due to the effects observed in the 90-day study on the mammary glands of the males, the following endpoint shall be investigated during the conduction of the extended one-generation reproductive toxicity study on 4,4'-sulfonyldiphenol:

- mammary glands development in P and F1 animals (modification of the architecture of the mammary glands, i.e. terminal buds and terminal ducts).

A histopathological analysis has to be performed as done by Delclos *et al.* in 2014. F1 animals (1 male and 1 female per litter per dose) will be euthanized at PND21. Furthermore, mammary glands of P and F1 animals (1 animal per litter per sex per dose) will be microscopically evaluated at the end of the experiment. Mammary gland histopathology should be conducted as described in OECD Guidance document 151, paragraphs 79-80.

In their comments on the draft decision and during the MSC meeting, the Registrant(s) agreed to perform the EOGRTS with the inclusion of Cohorts 2A, 2B and 3, and with the extension of Cohort 1B to include the F2 generation.

Conclusion

Therefore, pursuant to Article 46(1) of the REACH Regulation, the Registrant(s) are required to carry out the following study using the registered substance subject to this decision: Extended-one generation reproductive toxicity study in rats, oral route, with the developmental neurotoxicity and immunotoxicity (DNT/DIT) cohorts and with the conditional extension of Cohort 1B to mate the F1 animals to produce an F2 generation. Learning and memory test (The Morris water maze test is recommended) shall be performed in the developmental neurotoxicity cohort.

2. Toxicokinetics (test method: EU B.36/OECD 417)

The Concern(s) Identified

No toxicokinetics study is available. Currently, the assessment of toxicokinetics behavior of 4,4'-sulfonyldiphenol is based on physico-chemical data and on available toxicological studies.

Why new information is needed

An understanding of the toxicokinetics and the metabolism of 4,4'-sulfonyldiphenol is of major importance for the risk assessment as it enables quantification of the toxicokinetics relationship between the critical exposure in animal experiments and the corresponding exposure in humans.

Toxicokinetic results could explain some of the adverse effects seen in the toxicological studies. The 28-day repeated dose toxicity study in rats (OECD 407) showed some adverse effects in kidney and liver. In the urine, increased urobilinogen was observed, which may be due to damage of the liver or due to an increased hemolysis in the gastrointestinal tract (GIT). Moreover, it could help to elucidate the reasons for the extra medullary hematopoiesis.

Furthermore, UDP-glucuronosyltransferases (UGTs) isoforms metabolize Bisphenol A, which is structurally related to 4,4'-sulfonyldiphenol. Bisphenol A has shown to inhibit UGTs, with a stronger inhibition towards UGT2B isoforms than UGT1A isoforms. This might lead to an inhibition of Bisphenol A elimination or an inhibition of metabolism of endogenous substances, like testosterone (Jiang *et al.,* 2013). The toxicokinetics study could help to verify the mode of action of 4,4'-sulfonyldiphenol on its own metabolism.

Toxicokinetics results will also help to interpret data from the extended one-generation reproductive toxicity study.

In a PfA, it was asked to clarify the primary purposes for requesting this study. In another PfA the performance of the toxicocinetics study was supported to determine the optimum sampling times and other target tissues to be investigated in the comet assay. In a third PfA, the tiered approach (i.e. toxicokinetics to confirm the results of the in vivo micronucleus assay) proposed by the Registrant(s) was supported.

The Registrant(s) confirmed its position in their comments to PfAs: the test should be performed to identify the appropriate testing strategy for in vivo genotoxicity (tiered testing strategy). For the Registrant(s) the performance of a Toxicokinetics study only to identify the appropriate test design for the comet assay is not supported (no improved hazard identification and/or risk management measures).

For ECHA, the primary purposes of this study are:

- to have ADME data, to establish the mode of action of the substance as endocrine disruptor, in the context of the MoA/HRF (Mode of Action / Human Relevance Framework);
- to be able to interpret data from other toxicological tests by distinguishing the effect of the substance itself or its metabolites;
- to establish the half-life time of the substance in vivo;
- to establish the optimum sampling time for the comet assay (as it is the substance itself that induces clastogenic effects *in vitro*);
- to have tissue specific data on the bioavailability of the substance due to possible inhibitory activity on its metabolizing enzymes (UGTs).

Available *in vitro* data on the substance itself and other data support the need for ADME data for 4,4'-sulfonyldiphenol:

- The main metabolism pathway for Bisphenol A (BPA) in *Cynomolgus* monkeys and male rats is via glucuronidation. Thus, even if the excretion of the metabolite is different (monkeys excreted the metabolite via urine, while, in rats, it was found in the feces) (Kurebayashi *et al.*, 2002, 2003), the metabolism is similar in both species. Skledar *et al.* (2015) showed that 4,4'-sulfonyldiphenol is also glucoronised *in vitro*.

- Skledar et al. (2015) showed differences between glucuronidation of BPF, BPA, and 4,4'-sulfonyldiphenol in homologous human UGT enzymes. Whereas 4,4'-sulfonyldiphenol was mainly metabolized with UGT1A9, followed by UGT2A1. BPF was mainly metabolized by UGT1A10 followed UGT2A1 and BPA by UGT2A1 respectively. These differences in UGT activity for different bisphenols show the complexity of the metabolism of bisphenols and one toxicokinetic study of one analogue of this group cannot represent all the mechanisms of all other group members. Especially because the enzyme activity differs in tissues, with different enzyme affinity to the substrate, these enzymes have not the same metabolism (aspect of time and saturation) for the different bisphenols (Skledar et al., 2015).

- Furthermore, Skledar *et al.* (2015) showed also a higher enzymatic activity with enzymes of the human liver microsomes than with the human intestine microsome for 4,4'-sulfonyldiphenol.

- Court *et al.* (2010) and Ohno & Nakajin (2009) showed that different UGTs are expressed in different tissues and UGT1A9 is mainly expressed in the liver and the kidney.

- According to EFSA Opinion on BPA, unconjugated BPA is toxicologically relevant, because it is biologically active, whereas conjugated BPA is the biologically inactive form.

- Most of these results are produced *in vitro*. The aspect of different pathways in an organism and different exposure of different tissues shall be considered. Skledar *et al.* (2015) showed differences in UGT metabolism for different bisphenols. It is therefore not possible to use toxicokinetic data of BPA for the interpretation of the results and the conclusion on the mode of action for 4,4'-sulfonyldiphenol.

The results could inform about target tissues, which is particulary important for this substance, as it is a potential endocrine disruptor. The results could therefore also improve the design, the interpretation, and the reliability of the comet assay.

The generated data will be relevant for the human health as well as for the refinement of the DNELs.

Considerations on the test method and testing strategy

A toxicokinetic study (test method: EU B.36/OECD 417) shall provide the appropriate information. The study will indeed generate substance specific data on absorption, distribution, excretion and metabolization for 4,4'-sulphonyldiphenol. It will help to provide a relation of dose to the toxicity observed in other studies and it will help to understand the mode of action.

Since the majority of the toxicological studies on the registered substance are/will be performed in rats, ECHA is of the opinion that the scope of the toxicokinetics study will warrant study on rats. Moreover, according to the OECD 417, rat is the preferred species.

Concretely, the substance shall be administered once via the oral route (gavage) in the same vehicle as in the other toxicological studies (Paragraph 27 OECD TG 417). Furthermore, a pilot study shall be be conducted prior to the main study as explained in OECD TG 417 (Paragraph 20 and 21). Dose level shall be selected according the pilot study and for the main study two dose levels shall be selected.

The following tissues shall be collected, according to paragraph 37 of the TG OECD 417: Liver, fat, GI tract, kidney, spleen, whole blood, residual carcass.

Conclusion

Therefore, pursuant to Article 46(1) of the REACH Regulation, the Registrant(s) are required to carry out the following study using the registered substance subject to this decision: Toxicokinetics (test method: EU B.36/OECD 417).

ECHA recommends to perform the Toxicokinetics test prior to conducting the comet assay.

3. *In vivo* alkaline comet assay performed in rats by oral administration (gavage)(test method: OECD 489)

The Concern(s) Identified

In the registration dossier, the AMES tests (OECD 471)(1989, 1987, 1996, 1999, 2006, 1991) and mammalian cell gene mutation assay (OECD 476)(1990) were negative.

Positive results were found in two *in vitro* mammalian chromosome aberration studies (OECD 473)(1991, 1999) without S9 metabolic activation. In the first study (1991), positive results (significant number of cells with aberration) were found after 13h exposure duration and in the other study (1999), positive results (significant number of cells with aberration) were found after 24h exposure duration (negative after 6h treatment). Both tests were negative with S9 metabolic activation.

An *in vivo* mammalian erythrocyte micronucleus test (OECD 474)(2010) showed no micronucleus induction by 4,4'-sulfonyldiphenol in bone marrow. However, evidence of bone marrow exposure by the test substance has not been shown by the Registrant(s). Indeed, no decrease in the ratio of polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCE) (PCE/NCE ratio) has been observed in the treated animals compared to control animals during the evaluation of the micronucleus test. Furthermore, no plasma or blood analysis to check for the presence of the test substance have been performed. In addition, no toxicokinetics data are available to demonstrate bone marrow exposure.

Furthermore, in the literature, several concerns are raised on the genotoxic potential of 4,4'-sulfonyldiphenol: Lee *et al.* (2013) showed that 4,4'-sulfonyldiphenol can induce DNA double-strand breaks in mutant cells which lack the repair mechanism. Fic *et al.* (2013) showed that 4,4'-sulfonyldiphenol induces significant DNA damage after 24h exposure in an *in vitro* comet assay.

The 1991 *In Vitro* Mammalian Chromosomal Aberration Test (OECD TG 473) showed dose-related genotoxic effects of the parent substance in the absence of marked cytotoxicity.

The investigation of the direct sites of contact appears important to investigate the potential genotoxic effect of the parent substance, since it was the parent substance (and not its metabolites) that induced clastogenic effects in vitro.

Why new information is needed

The existing data are not sufficient to exclude any genotoxic potential of 4,4'-sulfonyldiphenol. Taking into account the high tonnage (1000-10000 T/year) and the uses of the substance, a risk for human health cannot be excluded. The genotoxic potential of the substance needs to be clarified. This could possibly lead to a classification for mutagenicity and related risk management measures.

Considerations on the test method and testing strategy

The *in vivo* alkaline comet assay shall be the method of choice to investigate further the uncertainties upon genotoxicity for the following reasons:

- The comet assay presents an increased sensitivity for detecting low levels of damage that might otherwise go undetected by the standard assays (Vasquez MZ, 2009 and Tice RR, 2000).
- The comet assay can detect single and double-stranded breaks, which can lead to chromosome aberrations.

- DNA damages can be tissue specific and the comet assay will allow investigation of several organs at the same time (Hartmann A, 2004).

In their comments to the draft decision, the Registrant(s) proposed an alternative testing strategy including the performance of a new *In Vitro* Mammalian Chromosomal Aberration Test (OECD TG 473) and determination of the bioavailability by an assessment of the plasma kinetics in the Toxicokinetics study (OECD TG 417) to validate the available Mouse Micronucleus Assay results. If nevertheless additional information on a further systemic target organ is requested, the Registrant(s) proposed to perform the comet assay on the liver only (and not on the other requested validated and non-validated organs).

With reference to the Registrant(s)' comment on the first draft decision, it was noted in a PfA that if the tokicokinetic study is performed in rats, this could not be used to demonstrate bone marrow exposure and validate the micronucleus test in vivo that was performed in mice. The Registrant(s) confirmed their position considering that in case sufficient bioavailability of the parent substance can be demonstrated in the plasma, exposure of the bone marrow in rodents would be demonstrated.

In another PfA, it was argued that the toxicokinetics study should be performed prior to the comet assay in order to establish the optimum sampling time(s).

In a third PfA, the tiered approach proposed by the Registrant(s) (i.e. toxicokinetics to confirm the results of the in vivo micronucleus assay) was supported.

Based on this PfA and the comments from the Registrant(s), ECHA agrees that a tiered approach is appropriate. Therefore, the in vivo comet assay shall not be performed if both of the following conditions are fulfilled:

1. The results of the toxicokinetics study demonstrate the presence of the parent substance in the plasma, and
2. It is demonstrated that the results of this in vivo toxicokinetics study in rats can be used to confirm the results of the available in vivo micronucleus study in mice. A justification shall be provided for the ability to use the rat data and any other available data to confirm the results of the mouse study and to demonstrate that the conditions specified in the OECD guidance 474 (adopted in 26 September 2014) regarding the target tissue exposure (paragraph 40) are fulfilled.

If these conditions are not fullfilled then the comet assay shall be performed as specified below.

In one PfA, it was suggested to add the duodenum/jejunum and to consider other tissues, i.e gonadal cells. The Registrant(s) in their response indicated the need of preliminary range-finding study for dose selection for every organ to be tested, in order to exclude DNA lesions due to cytotoxicity. Target tissue toxicity of the substance was indeed described in the liver and in the gastrointestinal tract. Considering the choice of organs, the Registrant(s) indicated that interpretation of data on validated organs (liver and glandular stomach) would be more reliable than assessment of non-validated organs.

ECHA considered the Registrant(s)' comments and still finds it appropriate to examine the the glandular stomach and duodenum[4] (as first sites of contact) as well as the liver, since positive results were seen in the chromosome aberration tests without metabolic activation. It is acknowledged that duodenum was not part of the validation performed by OECD/JaCVAM. However, ECHA notes that the duodenum is mentioned several times in the TG 489 and considers that duodenum is now routinely analysed by test laboratories performing the comet assay.

Therefore, at least the following tissues shall be investigated:

- glandular stomach and duodenum

Reasons: As set out in the OECD TG 489, the glandular stomach and duodenum are recommended as tissues to examine site of contact effects after oral exposure. Moreover, according to the test guideline, duodenum may be considered more relevant for humans. In view of the following possible variables: different tissue structure and function of the stomach and duodenum; different pH conditions; probable different absorption rates of the substance and possible breakdown product(s) between these two tissues; type of substance and its possible breakdown product(s), ECHA considers that it is necessary to sample both tissues to increase the reliability of the analysis of genotoxicity at the site of contact. Furthermore, dilated caecum and haemorrhage are shown in the intestinal tract during the 28-day repeated dose study.

and

- Liver

Reasons: As set out in the OECD TG 489, the liver is recommended as the primary site of xenobiotic metabolism, and an often highly exposed tissue to both parent substance and metabolites. Furthermore, the 28-day study showed disorders in the liver such as hepatocytes Hypertrophy.

The Registrant(s) are reminded that according to Annex IX, Section 8.4., column 2 of the REACH Regulation, if positive results from an in vivo somatic cell study are available, "the potential for germ cell mutagenicity should be considered on the basis of all available data, including toxicokinetic evidence. If no clear conclusions about germ cell mutagenicity can be made, additional investigations shall be considered".

The Registrant(s) may also consider examining gonadal cells when conducting the requested comet assay, as it would optimise the use of animals. In respect to possible outcomes ECHA notes on the one hand that a positive result in whole gonads is not necessarily reflective of germ cell damage since gonads contain a mixture of somatic and germ cells. However, such positive result would indicate that the substance and/or its metabolite(s) have reached the gonads and caused genotoxic effects. This type of evidence may be relevant for the overall assessment of possible germ cell mutagenicity including classification and labelling according to the CLP Regulation.

Conclusion

Therefore, pursuant to Article 46(1) of the REACH Regulation, the Registrant(s) are required to carry out the following study using the registered substance subject to this decision:

[4] ECHA considers that the duodenum is the most appropriate part of the intestine to be tested, as it is the first part of the intestine and directly connected to the stomach. The duodenum tissue sampled may contain a small part of the jejunum.

In vivo alkaline comet assay performed in rats by oral administration (gavage)(test method: OECD 489) on tissues as specified above (at least the liver, glandular stomach and duodenum). This request is part of a tiered approach as specified above.

4. Medaka or Zebrafish Extended One Generation Reproduction test (MEOGRT or ZEORGT)

The Concern(s) Identified

In vitro tests and *in vivo* studies show endocrine disrupting (ED) modes of action for 4,4'-sulfonyldiphenol (see above, under endpoint 1. of this section III). Some adverse effects were observed in rat studies, i.e. fertility concern in the Reproduction/Developmental Toxicity Screening Test, some adverse effects on endocrine organs in the Repeated Dose 90-Day Oral Toxicity Study (see above, under endpoint 1. of this section III).A concern for fish is also suspected. Indeed, published studies by Ji *et al.* (2013) and Naderi *et al.* (2014) have shown effects of 4,4'-sulfonyldiphenol on the sexual development and reproduction of zebrafish (Danio rerio). The authors reported among others, reduced body length and weight in males and increased malformation rates in exposed F1 embryos, reduced egg production and sperm count and a possible skewing of phenotypic sex ratio.

In conclusion, taking into account the high tonnage (1000-10000 T/year) and the uses of the substance, a risk for the environment cannot be excluded. Endocrine disruption in fish needs to be clarified.

Why new information is needed

In their comments, the Registrant(s) pointed out different weaknesses of both fish literature studies and proposed to perform a Fish lifecycle toxicity test (FLCTT) to enable a final conclusion on Endocrine disruption for the environment. The evaluating MSCA consulted the ED expert group and based on the discussion concluded that further testing for the environment was appropriate to clarify the ED concern.

Results of this fish study will allow to elucidate ENV ED adverse effects, which could lead to an identification of the substance as SVHC (ED for ENV) according to Art.57(f) and possible inclusion in Annex XIV of the REACH Regulation.

Moreover, as there are concerns for fish fertility and development (based on the 2 available literature studies), a long term fish study covering these endpoints is needed to allow a more accurate assessment of the risk for the environment.

Considerations on the test method and testing strategy

During the first commenting period, the Registrant(s) proposed to add a Fish Lifecycle Toxicity Test (FLCTT) to the draft decision. The evaluating MSCA agreed with the Registrant(s) and added this request to the decision that was sent to the Member States and ECHA for commenting.

Several PfAs were received on the FLCTT.

In one PfA, the request for the FLCTT was supported.

In another PfA, it was proposed to request a test according to OECD TG 240 (the Medaka Extended One Generation Reproduction Test), as it is fully validated and has a broader scope (covers the potential effect of a chemical in the stages of sexual development to reproduction periods of F1, not covered in the FLCTT).

In a third PfA, it was agreed that further testing is needed, but proposed to repeat an OECD TG 234 (Fish Sexual Development Test - FSDT) as it requires far fewer vertebrate animals than a FLCTT. Alternatively, further justification is requested to explain why a level 5 test (FLCTT) is necessary rather than a level 4 study (FSDT) (e.g. explaining whether there are reliable indications that transgenerational effects are of concern). Furthermore, as the Japanese Medaka extended one generation test is currently considered to be the most robust protocol for fish life cycle testing and in the absence of a validated test guideline for zebrafish, the guidance provided in the draft decision for the requested FLCTT with zebrafish was welcomed. In this PfA, a formal mechanism to allow the evaluating MSCA and/or ECHA to review, provide comments and/or amend the Registrant's test protocol before the study begins was asked. If this was not possible, then more detail of the study protocol should be appended to the decision (validity/test acceptance criteria, extend of gonad histology, chemical analysis requirements...).

The Registrant(s) in their comments to the PfAs argued that zebrafish is the preferred species, as endocrine concern for 4,4'-sulfonyldiphenol in fish originated from two available literature studies which were performed with this species. Moreover, the Registrant(s) argued that according to their knowledge only one commercial laboratory globally would have experience conducting MEOGRT (OECD TG 240).

The Registrant(s) supported conducting an OECD TG 234 if a negative result would satisfy the concern and no further test would be necessary. Furthermore, the Registrant(s) supported the proposal to establish a formal mechanism to allow the evaluating MSCA to review, provide comments and/or amend the FLCTT plan before the study begins rather than appending the decision with a detailed protocol.

After consideration of the different PfAs and the comments made by the Registrant(s), ECHA still considers that a level 5 test (OECD ED conceptual framework) is needed as effects were observed in Naderi *et al.*, 2014 (OECD TG 234, with deviations). If result of a newly performed OECD 234 would be negative, concerns would still remain and an OECD CF level 5 test would still be needed for final conclusion on potential ED properties.

Moreover, OECD guidance document N° 150 indicates that conduct of TG 234 (FSDT) would be particularly relevant if the test chemical is suspected to act primarily on the sexual development phase of the fish lifecycle (as opposed to the reproductive phase), because it provides apical information on phenotypic sex ratio which is fixed during the fry or juvenile stages of the species used in this test.

OECD guidance doc. N°150 indicates that some EDs may be more toxic to reproduction than to sexual development in which case OECD TG 234 (FSDT) would be less responsive than a lifecycle test. For this substance, fecundity and fertility are already affected at ≥ 0.5 µg/l (Ji et al., 2013), while effects on sex ratio were observed at 10 µg/l (58.8 %) and 100 µg/l (66.7 %) (Naderi et al., 2014) based on male, female determination. Intersex and undifferentiated animals were not determined. OECD TG 234 does not cover reproduction. Therefore a level 5 test is particularly appropriate. If there are in vivo alerts for potential ED effects and data showing ED MoA a MEOGRT is one of the possible studies for further ED testing. Moreover the MEOGRT is a fully validated OECD test guideline.

During MSC meeting the Registrant(s) confirmed that the preferred species is zebrafish for the reason mentioned above and that he has capability to perform the ZEORGT. They also argued that the FLCTT covers all the endpoints from the two literature studies: Naderi eat al, 2014 (similar to OECD 234) and Ji et al, 2013 (similar to OECD229). ECHA considers that the aim of this fish extended one generation study is not to replicate the 2 available literature studies. ECHA considers that all relevant endpoints should be considered as explained further below. Moreover, ECHA considers it necessary to investigate the effect of 4,4'-sulfonyldiphenol also on F1 reproduction and sexual development as evidence is provided for transgenerational reproductive abnormalities in fish (medaka) caused by developmental exposure to the structural analogue 4,4'-isopropylidenediphenol (BPA) (Bhandari et al, 2015).

Both MEOGRT and ZEOGRT cover this endpoint in addition to the endpoints covered in the FLCTT:

F0 MEOGRT: Fecundity, fertility and growth
F0 ZEORGT: Fecundity and fertility, survival, growth, VTG, 11-keto testosterone, sex ratio, histopathology (as specified in OECD TG 240, §49)
F0 FLCTT: Fecundity, fertility, growth, hatching success, time to 1st spawn, survival, VTG, gonad histopathology (sex ratio, intersex evaluation and gonad stage)

F1 MEOGRT: Hatching success, survival, growth, VTG, secondary sex characteristics, genetic sex determination, time to 1st spawn, fecundity, fertility, histopathology (as specified in OECD TG 240, §49)
F1 ZEOGRT: Hatchability (time to hatch and hatching success), survival and growth of the early life stage, juveniles and adults, reproduction (time to first spawning, fecundity, fertility), sex ratio, VTG, 11-keto testosterone, histopathology (as specified in OECD TG 240, §49)

F1 FLCTT: Hatching, survival, growth

F2 MEOGRT: Hatching
F2 ZEOGRT: Hatching success
F2 FLCTT: /

In addition less animals are used in the MEOGRT/ZEORGT than foreseen in the FLCTT (924/1104 compared to 1740). The Registrant(s) proposed to lower the number of fish and the number of replicates in the FLCTT. As this will lower the statistical power, ECHA cannot agree with this option.

However given the preference to test zebrafish and the concern expressed by the Registrant(s) regarding laboratory capacity for OECD 240 testing using medaka, ECHA is leaving the possibility open for the Registrant(s) to adapt the MEOGRT to the ZEOGRT. In the OECD TG 240, it is indeed stated that "the specific methods and observational endpoints detailed in this guideline are applicable to Japanese medaka alone. Other small fish species (e.g. zebrafish) may be adapted to a similar test protocol".

If the ZEOGRT is the choice of the Registrant(s), the test shall be performed according to the appended ZEORGT protocol. Any deviation from the protocol and deviation from the acceptance criteria, and considerations of potential consequences on the outcome of the test need to be justified and reported. Any foreseen deviation for technical reasons are to be agreed with the evaluating MSCA.

If the test is performed with zebrafish, it shall include measurement of 11-keto-testosterone, to elucidate the possible androgen activity (see above, under endpoint 1.of this section III).

In case the test is performed with medaka, the OECD 240 shall be followed.

The Registrant(s) questioned if, in the event of a negative outcome with medaka, further testing would be needed due to the biological differences between the two species. ECHA considers it not necessary to ask further testing in case the MEOGRT would be conducted and the result would be negative.

A range finder study shall in addition be performed (in accordance to OECD TG 210, Early life stage toxicity) to determine the concentration range to be used in the MEOGRT/ZEORGT.

In another PfA, it was recommended that "The maximum test concentration should be 10% of the LC50 from the OECD TG 210 range finding study or 10 mg/L whichever is the lower".

The Registrant(s) in their comment argued that this is appropriate in the absence of other data. However a 75d NOEC growth >100 µg/l is available (Naderi et al., 2014). Furthermore when evaluating endocrine properties systemic toxicity should be avoided (Wheeler er al, 2013) and growth is a clear indication thereof. Therefore the 32d NOECgrowth resulting from the OECD210 range finding study is considered appropriate as the highest concentration in the FLCTT. The Registrant(s) also stipulated that the test concentration should go as low as 0.5µg/l to cover the effect level identified in the study of Ji et al., 2013, but in that case the recommended spacing factor of 3.2 cannot be used.

ECHA considered the PfA and the comment made by the Registrant(s) and agrees that any confounding impact of systemic toxicity should be avoided. In Naderi et al., 2014, increased mortality (~30%) was observed at 100 µg/L. At 10 mg/L, systemic toxicity is therefore highly expected. Taking into account that growth can also be affected by endocrine mode of action, the test concentrations in the final study should be based on the results of the range finding study. ECHA notes that the use of the NOEC growth from the range finding study as the highest concentration for the final study would not allow to evaluate all the ED endpoints, therefore the LOEC growth is recommended to be used as highest concentration. It is not excluded that the spacing factor of 3.2 could be extended to maximum 5, if necessary, to reach the dose as low as 0.5 µg/l when 5 concentrations are tested.

Therefore ECHA considers the MEORGT or ZEORGT as most appropriate study to elucidate ENV ED adverse effects and to allow a more accurate assessment of the risk for the environment.

Conclusion

Therefore, pursuant to Article 46(1) of the REACH Regulation, the Registrant(s) are required to carry out the following study using the registered substance subject to this decision: Medaka Extended One Generation Reproduction Test (MEOGRT) (according to OECD 240) or Zebrafish Extended One Generation Reproduction Test (ZEORGT) (according to the appended protocol) including a range finding study, as specified in the annex 2 to this decision.

5. Exposure data and exposure assessment

The Concern(s) Identified

In the registration dossier, no exposure assessment was conducted based on the conclusion of the Registrant(s) that "*no hazard was identified*". Only Use Descriptors information (SU-Sector of end use, PC-Chemical product category, PROC-Process category, ERC-Environmental release category, AC-Article category) were provided.

However, adverse effects caused by the substance have been observed for both human health and environmental endpoints and in particular:

- in the 28-day study (OECD TG 407) (LOAEL=200 mg/kg bw/d; bw gain, urinalysis, kidney weight, histological changes in the caecum)
- in the OECD 421 study (fertility concerns at 300 mg/kg bw/d)
- in fish studies (Ji *et al.*, 2013 and Naderi *et al.*, 2014, with reproductive adverse effects from 0,5 µg/L in Ji *et al.*).

Furthermore, the oestrogenic activity is relevant for many species and might therefore constitute a risk for many species, depending on the exposure.

Additionally, there is evidence of consumer exposure.

4,4'-sulfonylbisphenol is produced or imported at high tonnages. It has been reported to be used in thermal paper (such as cash register receipts) and in can linings and plastics for food storage (it has been found to migrate into food). The substance has been identified in indoor dust and has been detected in 81% of tested urine samples from New York and seven Asian countries (see more details and references at the following link: http://oehha.ca.gov/multimedia/biomon/pdf/110812Bisphenols.pdf).

Moreover, based on the results of the available screening test (OECD 301C, modified MITI), 4,4'-sulfonylbisphenol is not readily biodegradable. No degradation was seen in seawater (TOC Handai method and sea river die-away). The persistency adds therefore to the concern.

Why new information is needed

The available information did not enable the evaluating MSCA to quantify the risk for the registered substance, despite some concern for human health and the environment. In order to clarify this concern further information on exposure shall be submitted, as developed below.

Considerations on the approach to generate the information

Exposure data and exposure assessment for all life cycle steps shall be provided in accordance with chapters R12 to R18 of the Guidance on information requirements and chemicals safety assessment (Exposure assessment)[5]

Exposure scenarios shall be described with appropriate levels of details to allow a correct appraisal of exposure to industrial and professional workers, consumers and environment (water, soil).

[5] ECHA, Guidance on information requirements and chemical safety assessment. Chapter R.12, R.13, R.14, R.15, R.16, R.17 and R.18

The different uses of the substance shall be explicitly described (monomers, intermediate, process regulator etc.).

The type of polymer shall be clarified (e.g. polysulfone, polyethersulfone, etc.). Information on a potential release of the substance from the polymer matrix shall be provided.

More details on the kind of articles shall be given (i.e. specify in detail which articles/products are covered by the different Article category and Chemical product categories).

Industrial, professional and consumer use of articles containing 4,4'-sulfonylbisphenol shall be provided. The amount of the substance in the article shall be specified.

Article service life (including recycling and waste) as well as possible release of the substance from the articles shall be considered.

Moreover, the tonnage distribution per use shall be estimated.

In their comments on the draft decision, the Registrant(s) agree to provide the requested data.

Conclusion

Therefore, pursuant to Article 46(1) of the REACH Regulation, the Registrant(s) are required to provide exposure data and exposure assessment for all life cycle steps for the registered substance subject to this decision, in accordance with chapters R12 to R18 of the Guidance on information requirements and chemicals safety assessment (Exposure assessment) with appropriate level of details, as explained above.

IV. Adequate identification of the composition of the tested material

In relation to the required experimental stud(y/ies), the sample of the substance to be used shall have a composition that is within the specifications of the substance composition that are given by all Registrant(s). It is the responsibility of all the Registrant(s) to agree on the tested material to be subjected to the test(s) subject to this decision and to document the necessary information on composition of the test material. The substance identity information of the registered substance and of the sample tested must enable the evaluating MSCA and ECHA to confirm the relevance of the testing for the substance subject to substance evaluation. Finally, the test(s) must be shared by the Registrant(s).

V. Avoidance of unnecessary testing by data- and cost-sharing

In relation to the experimental stud(y/ies) the legal text foresees the sharing of information and costs between Registrant(s) (Article 53 of the REACH Regulation). Registrant(s) are therefore required to make every effort to reach an agreement regarding each experimental study for every endpoint as to who is to carry out the study on behalf of the other Registrant(s) and to inform ECHA accordingly within 90 days from the date of this decision under Article 53(1) of the REACH Regulation. This information should be submitted to ECHA using the following form stating the decision number above at: https://comments.echa.europa.eu/comments_cms/SEDraftDecisionComments.aspx

Further advice can be found at http://echa.europa.eu/regulations/reach/registration/data-sharing.

If ECHA is not informed of such agreement within 90 days, it will designate one of the Registrants to perform the stud(y/ies) on behalf of all of them.

VI. Information on right to appeal

An appeal may be brought against this decision to the Board of Appeal of ECHA under Articles 52(2) and 51(8) of the REACH Regulation. Such an appeal shall be lodged within three months of receiving notification of this decision. Further information on the appeal procedure can be found on the ECHA's internet page at http://www.echa.europa.eu/regulations/appeals. The notice of appeal will be deemed to be filed only when the appeal fee has been paid.

Authorised[6] by Leena Ylä-Mononen, Director of Evaluation

Annex 1: List of registration numbers for the addressees of this decision. This annex is confidential and not included in the public version of this decision.

Annex 2: Protocol for ZEOGRT

[6] As this is an electronic document, it is not physically signed. This communication has been approved according to ECHA's internal decision-approval process.

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Annex 2:

PROTOCOL FOR ZEOGRT

A Zebrafish Extended One Generation Reproduction Toxicity (ZEOGRT) test covers population relevant endpoints and will enable to conclude on endocrine disruption for the environment.

The test design is an adaptation of the test protocol of OECD 240 (Medaka Extended One Generation Reproduction test). In the OECD TG 240, it is stated that "the specific methods and observational endpoints detailed in this guideline are applicable to Japanese medaka alone. Other small fish species (e.g. zebrafish) may be adapted to a similar test protocol".

The test is started by exposing sexually mature males and females (at least 15 wpf) for 6-8 weeks. The effect of the test substance on reproduction (fecundity and fertility) of the parental F0-generation shall be evaluated. At the end of the F0-exposure period survival, growth and sex ratio shall be examined. Upon sacrifice, the adult F0 fish gonads will undergo histopathological evaluation to identify phenotypic sex and levels of 11-keto testosterone and the biomarker vitellogenin will be measured in all individuals.

As near as possible to the first day of the fourth week, eggs are collected to start the F1 generation. F1 shall be initiated with 36 eggs per replicate to examine post-hatch survival. F1 shall then be reduced to 20 randomly selected fish per replicate to examine juvenile growth and reproduction (fecundity and fertility). During rearing of the F1 generation (a total of 17 weeks), hatchability, survival and growth, first time to spawn, gonad histopatholgy, sex ratio and levels of VTG (in males and females) and 11-keto testosterone (in males) are assessed. F1 generation shall be exposed in the same corresponding test concentrations as F0.

An F2 generation is started after the sixth week of the reproduction assessment and reared until completion of hatching.

Test method:

The test conditions provided in table 2 and exposure and measurement endpoint timelines provided in table 3 are to be followed.

The endpoints to evaluate are given in table 1.

Gonad histopathology in F0 and F1 should be performed according to OECD GD 123: Guidance on the diagnosis of endocrine–related histopathology in fish gonads.

In zebrafish there is a wide variation of sex ratios among different families and a strong influence from parental genotypes (Liew et al, 2012). As sex ratio may differ under specific conditions and with different genetic predispositions, differences within the batches in the ZEOGRT shall be overcome by :

- Randomization of group composition
- Group spawning and taking eggs from the group pool
- Statistical evaluation of the 4 replicates of spawning groups per concentration

Table 1: Endpoint overview of the ZEOGRT:

Time of exposure	Fish age	Phase	Course	Endpoints*
0 d	approx. 15 weeks	Reproduction F_0 generation	Start with spawning groups 5 male/5 female fish	**Total egg no/ day/ female (Fecundity) Fertilization rate (Fertility) (Cumulative egg no.)**
21 d	0 d	Fish, early life stage toxicity (FELS) F_1-generation	Start with 36 fertilized eggs per vessel (2x18 eggs in stainless steel fry cages)	**Time to hatch Hatching success**
24 d	3 d		Begin of hatch (hatch completion between 4 to 6 dpf)	
27 d	6 d		Feeding with breeding food *ad libitum*	
			Swimming up	
5 w	14 d		Feeding with *Artemia salina* (Lifefood)	
6 w	21 d		Photographic determination of survival; Transfer to main aquaria	**Post-hatch survival**
8 w	35 d		Photographic determination of length and survival Random reduction to 20 individuals	**Post-hatch survival Length**
		F_0-generation	Termination	**Length and weight at test termination Sex ratio Gonad histopathology (e.g. maturation stage; endocrine-related histopathology) Vitellogenin content in females and males 11-keto testosterone content in males**
12 w	63 d	Juvenile growth F_1-generation	Photographic determination of length and survival	**Survival Length Pseudo-specific growth rate**
13 - 18 w	70 - 105 d	Reproduction F_1-generation	Introduction of spawning trays Daily evaluation of egg numbers and fertilization rates	**Time to first spawning**
15 - 20 w	84 d - 119 d			**Total egg no/ day/ female (Fecundity) Fertilization rate (Fertility) (Cumulative egg no.)**
20 w	0 d	F_2-generation	Start with 20 fertilized eggs per vessel (in stainless steel fry cages)	**Time to hatch Hatching success**
	96 h		Hatch	
20 - 22 w	119 - 133 d	Test termination F_1-generation	End of F_1-generation	**Length and weight at test termination Sex ratio Gonad histopathology (e.g. maturation stage; endocrine-related histopathology) Vitellogenin content in females and males 11-keto testosterone content in males**

* Only the most relevant endpoints for endocrine effects were listed. Furthermore, behavioural abnormalities (e.g. orientation in the water body, food consumption, increased / decreased motility) could be observed during the whole time course of the study.

Table 2: Test conditions for the ZEOGRT:

GLP	Yes
Test species	Zebrafish (*Danio rerio*)
Test type	Flow through
Water temperature	27 +/- 2 °C
Illumination quality	Fluorescent bulbs (wide spectrum and ~150 lumens/m2) (~150 lux).
Photoperiod	12 h light, 12 h dark
Chamber size	20 – 25 L
Volume exchanges of test solutions	minimum of 5 daily
Acclimation period	at least two weeks prior to the test
Age of test organisms at start of exposure	adult fish, spawners (F_0-generation), approx. 15 weeks
Loading rate per replicate	F_0: 10 fish (5 males, 5 females) F_1: initiated with 36 eggs, reduced to 20 fish for juvenile growth and reproductive phase (randomised) F_2: 20 eggs (hatch only)
Loading rate per treatment	F_0: 40 fish F_1: initiated with 144 eggs, reduced to 80 fish for juvenile growth and reproductive phase (randomised) F_2: 80 eggs (hatch only)
Volume exchanges of test solutions	Minimum of 5 volume renewal/day
Number of treatments	5
Number of replicates per treatment	4
Feeding regime	Fry food (dry), live food (nauplii of *Artemia salina*), flake food
aeration	None unless oxygen concentration falls below 60 % saturation
Dilution water	reconstituted tap water
Test substance exposure duration	20-22 weeks
Test concentration	Since the acute fish toxicity of BPS exceeds 100 mg/L, the long term systemic toxicity effects on zebrafish reported in Naderi et al.(2014) and Ji et al. (2013) are considered in selecting appropriate test concentrations. Naderi et al.(2014) reported growth effects in male fish at 100 ug/L in a 75 day exposure whereas Ji et al. (2013) reported indications of toxicity at ≥0.5 ug/L in a shorter 21-day exposure. The published studies are not a sufficient basis to define exposure concentrations for the FLCTT because of the large discrepancy in the exposure period and magnitude of reported effect concentrations. - An appropriate high concentration will be determined in a fish early life stage range finding study in accordance with OECD210. Range finder test concentrations: 320, 100, 32, 10, 3.2 µg/L This covers the concentration range from Naderi et al.(2014) and the high and mid concentrations from Ji et al (2013). The highest range finder concentration exceeds those in the published studies. The exposure period (approx. 32-days) is longer than in Ji et al (2013). This will allow to ensure that a LOEC and NOEC are identified in this range finder. Based on the results of the range finding study, the LOEC growth is recommended as the highest test concentration. The concentration range should at least go as low as 0.5µg/L, if possible, to cover the LOEC reported in Ji et al. (2013). - Spacing factor : The spacing factor should be preferably 3.2 if possible, but could be extended to maximum 5.
Biological endpoints	F_0: Reproduction (Fecundity and Fertility), survival, growth, sex ratio, histopatholgy, Vitellogenin, 11-keto Testosterone F_1: Hatching success Survival, growth (Early life stage, juveniles and adults) Reproduction (Time to first spawning, Fecundity and Fertility) Sex ratio Vitellogenin, Histopathology, Optional: 11-keto Testosterone F_2: Hatching success

Determination other parameters like pH, dissolved oxygen, t°, ...	Start and end of exposure (in dilution water supply): total hardness, acid capacity, TOC Once a week : flow rate in each test vessel Twice weekly: pH (alternating replicates), dissolved oxygen (all replicates) Daily: Instantaneous temperature (alternating replicates) Continuous temperature measurement with a data logger in one control replicate. Frequency of measurement may be increased if needed.
Test acceptability criteria for controls/ validity criteria	Dissolved oxygen ≥ 60 % Mean water temperature: 27 ± 2 °C Successful reproduction in controls: at least 10 eggs per female and day, 80 % fertility Post hatch survival (larvae), controls: ≥ 75 % Survival of juveniles and adults, controls: ≥ 90 % Sex ratio preferably between 30 % to 70 %

Table 3: Exposure and measurement endpoint timelines for the ZEOGRT