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

For biphenyl, CAS No 92-52-4 (EC No 202-163-5)

Addressees: Registrant(s)[1] of biphenyl (Registrant(s))

This decision is addressed to all Registrant(s) of the above substance with active registrations on the date on which the draft for the decision was first sent for comments, with the exception of the cases listed in the following paragraph. A list of all the relevant registration numbers subject to this decision is provided as an annex to this decision.

Registrants holding active registrations on the day the draft decision was sent for comments are *not* addressees of this decision if they are: i) Registrant(s) who had on that day registered the above substance exclusively as an on-site isolated intermediate under strictly controlled conditions and ii) Registrant(s) who have ceased manufacture/import of the above substance in accordance with Article 50(3) of Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH Regulation) before the decision is adopted by ECHA.

Based on an evaluation by Portuguese Environment Agency as the Competent Authority of Portugal (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 28 June 2014, 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 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 Portugal has initiated substance evaluation for biphenyl, CAS No 92-52-4 (EC No 202-163-5) 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.

On the basis of an opinion of the ECHA Member State Committee and due to initial grounds

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

for concern relating to potential PBT properties and high aggregated tonnage, biphenyl was included in the Community rolling action plan (CoRAP) for substance evaluation to be evaluated in 2013. The updated CoRAP was published on the ECHA website on 20 March 2013. The Competent Authority of Portugal was appointed to carry out the evaluation.

In the course of the evaluation, the evaluating MSCA noted an additional concern for reproductive toxicity.

The evaluating MSCA considered that further information was required to clarify the above mentioned 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 17 March 2014.

On 29 April 2014 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 commenting phase

By 5 June 2014 ECHA received comments from the Registrant(s) of which it informed the evaluating MSCA without delay. By 26 June 2014 Registrant(s) submitted update(s) of the registration dossier(s).

The evaluating MSCA considered the comments received from the Registrant(s).
On basis of this information, only the deadline in Section II was amended. The comments were reflected in Section III of the draft decision (Statement of reasons).

Commenting by other MSCAs and ECHA

In accordance with Article 52(1) of the REACH Regulation, on 5 March 2015 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 10 April 2015 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 20 April 2015 ECHA referred the draft decision to the Member State Committee.

By 11 May 2015, in accordance to Article 51(5), the Registrant provided comments on the proposal(s) for amendment. The Member State Committee took the comments of the Registrant on the proposal(s) for amendment into account.

After discussion in the Member State Committee meeting on 8 to 11 June, a unanimous agreement of the Member State Committee on the draft decision as modified at the meeting was reached on 11 June 2015. 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. Ready biodegradability (test method: Closed bottle test, OECD 301D);

2. Sediment simulation testing (test method: Aerobic and anaerobic transformation in aquatic sediment systems, EU C.24./OECD 308) at a temperature of 12 °C, using Carbon 14 ring-labelled test substance, including identification of degradation products; this study shall be conducted only if results of the ready biodegradability test indicate that the registered substance screens as persistent/very persistent;

3. Extended one-generation reproductive toxicity study in rats, oral route (test method: OECD 443, including Cohort 2A and 2B, and Cohort 3).

The Registrant(s) should expand the study design to include the extension of cohort 1B to produce the F2 if, before the time of the testing, new information on uses leading to significant exposure of professionals or consumers according to criteria column 2 section 8.7.3 of Annex X is known to the Registrant(s). The justification for the extension must be included.

Pursuant to Article 46(2) of the REACH Regulation, the Registrant(s) shall submit to ECHA by **08 April 2018** from the date of the decision an update of the registration(s) containing the information required by this decision, including robust study summaries and, where relevant, an update of the Chemical Safety Report.

III. Statement of reasons

Based on the evaluation of all relevant information submitted on biphenyl and other relevant 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.

The information requested in Section II above constitutes the first tier in a testing strategy to clarify the concerns for potential PBT properties of the registered substance and also the additional concern, identified in the course of the evaluation, for reproductive toxicity. Hence, the evaluating MSCA will review the information submitted by the Registrant(s) as an outcome of tier 1 of the testing strategy, and evaluate if further information should be requested in order to clarify the concern for PBT properties.

Based on the first tier results and a conclusion on the Persistence property, a second tier of the PBT testing strategy may be triggered. Further information on ecotoxicity and bioaccumulation may be requested.

The evaluating MSCA will subsequently review the information submitted by the

Registrant(s) and evaluate if further information should be requested in order to clarify the PBT properties or for risk assessment.

1. Ready biodegradability study

Information on ready biodegradability is necessary in order to assess the properties of the substance and to decide whether it is persistent in relation to the criteria for PBT assessment (REACH Regulation, Annex XIII). To conclude on this concern it is considered appropriate that ready biodegradation screening is performed ahead of further simulation testing.

The information in the registration dossier concerning screening of biodegradation includes several studies, all with reliabilities assigned by the Registrant(s) as Klimisch 2 or higher.

From the "[redacted]" the results of a modified MITI test I (no GLP) were reported as key study, assigned Klimisch 2. The information on the test method for this study refers to the general methodology for the chemicals assessed under the referred database, which was considered to be similar to the 301C-Ready biodegradability by the Registrant(s). However, there was no adequate description of the procedure neither of the test conditions under which the study has been carried out. In this study, 66% BOD was observed after a 14 days incubation period at a level significantly exceeding the water solubility of the substance. A very short [redacted] study report and it's translation have been provided by Registrant(s), in the commenting phase, that includes only one set of final percentage figures and does not include additional raw information (i.e. figures, tables or graphs) in order to compare them and confirm degradation kinetics.

Therefore, based on the short translation from a [redacted] report, Ready biodegradability cannot be concluded nor confirmed from the information provided by Registrant(s) on the summary of this assay.

A supporting study (1994), conducted under method similar to EU Method C.5. Degradation Biochemical Oxygen Demand reports a 5-day BOD of 67% and 48.8% of the theoretical oxygen demand for non-acclimated and acclimated seeds, respectively. It seems that similar degradation percentages are obtained for non-acclimated and acclimated organisms, with very different volume of inoculum (x20) used as seed of acclimated (0.05 mL) and non-acclimated (1 mL). A lower concentration is referred regarding the acclimated seed, but no other information is provided, i.e. cellular density. Therefore, the yield of both systems cannot be compared and no conclusion can be made. This assay does not allow a conclusion on Ready biodegradability.

Other two supporting studies (1983) reported the results of: a) equivalent or similar to OECD Guideline 302 A - SCAS test (Inherent Biodegradability) (no GLP) with pre-adapted activated sludge microorganisms, showing > 95% primary biodegradation within 24 hours and b) a Sturm test (CO_2 evolution) in which pre-adapted activated sludge microorganisms were exposed to 20 mg/L biphenyl for 43 days using acetone as solvent. Monitoring of CO_2 evolution indicated that biphenyl is ultimately degraded by 88% after 43 days and 69% after 28 days. Both studies can be considered supporting on potential biodegradability but not on ready biodegradability.

In their comments to the initial draft decision the Registrant(s) considered that an Aerobic Mineralisation in Surface Water study (1980) and its publication (1983) are additional supporting information on rapid biodegradability. The test methodology was considered by the Registrant(s) similar or equivalent to OECD 309. The degradation results obtained in

this study performed at 20ºC, for three runs with water sampling at different times of the year (December, March and May), for the nominal concentration of 1 µg/L, are 61%, 76% and 78% (CO_2 evolution) in 30 days. Deviations from the standard test were also observed: concentration of suspended matter varied in the water samples depending on the period of the year and the release of a dam upstream of the sampling site, which increased the average value for the number of CFU/mL ranged from 3-11× 10^3 units/mL. According to the information included in the report replicates were not available for all days of sampling, and the material balance for the three different runs are deviating from standard conditions and validation criteria (40% and 78% in one run with replicate, and 82% and 95% in the other runs). The study can be considered to support the degradation of biphenyl under environmental conditions but ready biodegradability according to standard guidelines cannot be concluded.

Based on an evaluation of the available information, no conclusive assessment on Ready biodegradability of biphenyl is possible from the information provided by Registrant(s). Therefore, additional testing is requested in order to make a definitive conclusion on this endpoint based on reliable information. The request for a screening test on ready biodegradability would clarify this concern avoiding further simulation testing or indicating the need of further investigations.

In their comments the Registrant(s) propose that if a new study is required, the choice of one of the OECD 301 Ready Biodegradation should be left up to the Registrant(s). However, it is considered that the request should specify the ready biodegradability test to be conducted.

In their comments on proposals for amendment, the Registrant(s) agreed with one MSCA proposal to remove all degradation testing requests and alternatively with another proposal from one MSCA to add a new tier in the testing strategy for persistency, to perform an inherent biodegradability test if the substance is not ready biodegradable.

Additionally, the Registrant(s) proposed changes in the tiered strategy, in which the ready biodegradability test would be performed as an enhanced test, and an additional step would be added to perform an inherent biodegradability test if the substance fails the enhanced ready biodegradability test. The identification of the degradation products would be done in the sediment simulation test, if the substance screens as P.

It is considered that the proposed inherent biodegradability test is not necessary since the option of an enhanced test has been included in the decision.

Taking into account the physicochemical properties of the registered substance (volatility of 1.19 Pa and water solubility of 7.35 mg/L, at 25°C) and according to the REACH Guidance Document (Guidance on information requirements and chemical safety assessment, Chapter R.7b: Endpoint specific guidance) and the test guideline on biodegradation (OECD 301, Ready Biodegradability, adopted 17 July 1992), Closed Bottle test is adequate. In several biodegradation studies included in the registration dossier volatility losses were referred.

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: Ready biodegradability (test method: Closed bottle test, OECD 301D).

If the substance does not fulfil the criteria for Ready Biodegradability according to the OECD 301D guideline (OECD 301, Ready Biodegradability, adopted 17 July 1992), the Registrant(s) can consider to prolong the test with a duration up to 60 days, according to the REACH Guidance Document (Guidance on information requirements and chemical safety

assessment, Chapter R.7b: Endpoint specific guidance). If sufficient degradation is shown in such a test, i.e. the pass level is reached, the result is used to indicate that the substance will not persist in the environment and a sediment simulation test (point 2, section II) is considered not necessary.

However, ECHA highlights that if the result of the enhanced screening test does not allow the Registrant(s) to exclude unequivocally the persistence property, the Registrant(s) should carry out the simulation testing on degradation to conclude on P or vP (definitive criteria on the persistence).

2. Sediment simulation testing

Information on biodegradation is required in order to assess the properties of the substance and to decide whether it is persistent (P/vP) in accordance with Annex XIII of the REACH Regulation. Therefore, if it is concluded that the substance screens as persistent/very persistent, information on the simulation test in sediments is needed to address the suspected concern (PBT/vPvB properties).

The information in the registration dossier concerning sediment simulation testing includes two studies performed under GLP on aerobic biodegradation of biphenyl in water sediment/systems (1988). In the first study, the biodegradation of biphenyl was examined during 10 days in a natural river water/sediment system with naturally present microorganisms. Analysis of trapped $^{14}CO_2$ indicates ultimate biodegradation of 38.5% at 1 mg/L (volatility losses were 4.3-8.6% of total ^{14}C activity) and 42.4% at 0.077 mg/L (volatility losses were 4.6-5.9% of total ^{14}C activity). However for the active ecocores sacrificed at day 2, biphenyl volatility losses were higher accounting for 34.1% of total ^{14}C activity at 1 mg/L and 28.5% at 0.077 mg/L and no further analysis or justification was presented in the study report. In this study the half-life for biphenyl was determined to be 2-3 days for primary biodegradation.

In the second aerobic study the biodegradation of biphenyl was examined during 10 days in a natural lake water/sediment system with naturally present microorganisms. Analysis of trapped $^{14}CO_2$ indicates ultimate biodegradation of 17.7% at 1 mg/L and 37.8% at 0.077 mg/L. The half-life of biphenyl was estimated to be 6-10 days in the system. For the active ecocores sacrificed at day 10, biphenyl volatility losses accounted for 5.7-49.8% of total ^{14}C activity at 1 mg/L and 5.0-9.3% at 0.077 mg/L. Biphenyl volatility losses before acidification were 2.7-5.6% at 1 mg/L and 2.6-3.1% at 0.077 mg/L but volatility that occurred in ecocores sacrificed at previous sampling times were not specified in the dossier.

These studies assigned in the dossier as Klimisch 2, were considered by the Registrant(s) as equivalent or similar to OECD 308 test guideline and a weight of evidence approach was proposed concluding on a half-life on sediments of 10 days at 22°C. However, several limitations were identified in both studies: slightly lower water:sediment ratio; not fully conducted in the dark but with a photoperiod of 12h light:12h dark; volatility losses; low number of ecocores replicates; shorter test duration of 10 days instead of 28 days; material balance of the system under 90% in the river water/sediment study.

The photoperiod indicated in both studies is considered relevant since a DT_{50} photolysis of 19h is stated in the dossier, therefore, high disappearance (c.a. 31%) can be expected under each 12-hour light photoperiod applied in the tests. At the end of the test the ultimate biodegradation achieved was c.a. 40% on the river water/sediment test and 18% and 38% (depending on the concentration used) on the lake water/sediment test. Estimations for ultimate degradation half-life were not provided.

In their comments the Registrant(s) consider that while the two sediment simulation studies were not carried out for a long enough duration to directly calculate a mineralization half-life for comparison with the Annex XIII criteria, the results for primary degradation and ultimate biodegradation provide a very strong weight of evidence that the 120 or 180 day half-life criteria for fresh or marine sediment persistence would not be exceeded.

In another sediment and water study (1988), Klimisch 2 assigned by the Registrant(s), anaerobic biodegradation of biphenyl was examined during 12 weeks in a water/sediment system obtained from a sewage lagoon. Analysis of trapped $^{14}CO_2$ and $^{14}CH_4$ indicated no significant biodegradation (0%) of biphenyl via methanogenic or denitrifying processes. The study was considered by the Registrant(s) equivalent or similar to OECD Guideline 308, however, not enough information is provided to confirm this point.

According to the Registrant(s), anaerobic biodegradation is not expected to play an important role in the elimination of biphenyl from natural sediment/water systems. However it is considered that the anaerobic biodegradation could be relevant for risk assessment purposes, but not for persistency assessment.

Lack of information on these studies and the potential deviations on test procedures do not allow to conclude on the degradation in sediments. This study is considered as additional information indicating potential lack of biodegradability of biphenyl under anaerobic conditions.

Although the information available in the registration dossier from simulation tests indicates low half-lives (approximately 3 days in water and 10 days in sediments), different results were obtained in a simulation study with a half-life of 333 days in sediment (Pruell & Quinn, 1985). In this study, surface sediments were collected from locations along a polycyclic aromatic hydrocarbons (PAHs) polluted gradient in a bay and tested over a period of 394 days in controlled seawater mesocosms with controlled 27 days turnover time and simulated turbulence. The calculated half-life for biphenyl in sediment was 333 days. According to Registrant(s) comments, this study should not be considered for the assessment, since it fails to provide appropriate controls, standard experimental measurements and adequately provide conclusive results. Although it is recognised that the study can be considered unreliable for the determination of biphenyl half-life this information can be considered as supporting regarding the uncertainties on the biodegradability in sediments.

It is considered that the referred studies' limitations prevent to conclude on the environmental behaviour of the substance on sediment systems. Therefore, if the results of the ready biodegradability test indicate that the registered substance screens as persistent/very persistent a sediment simulation test shall be performed to remove uncertainties on persistence.

In their comments on proposals for amendment, the Registrant(s) proposed that the sediment simulation test would be performed without the anaerobic test.
However it is considered that the sediment simulation test should include all components of the test system in accordance with the test guideline OECD 308 that foresees both anaerobic and aerobic conditions.

ECHA took note of the Registrant(s) comments on the inclusion of the identification of degradation products in the sediment simulation test and points out that this identification is part of the standard guideline OECD 308.

The sediment simulation test shall be performed at a temperature of 12 °C (285 K) since this temperature is indicated in the Guidance on information requirements and chemical

safety assessment, Chapter R.11.(version 2.0, November 2014) as the EU average environmental temperature, to be used in the chemical safety assessment including PBT assessment.

Pursuant to Article 46(1) of the REACH Regulation the Registrant(s) shall submit the following information regarding the registered substance subject to the present decision: Sediment simulation testing (test method: Aerobic and anaerobic transformation in aquatic sediment systems, EU C.24/OECD 308) at a temperature of 12 °C, if results of the ready biodegradability test indicate that the registered substance screens as persistent/very persistent.

3. Reproductive Toxicity study in rats

The Commission Regulation (EU) 2015/282 of 20 February 2015 amending Annexes VIII, IX and X to REACH Regulation establishes that new substances, and those already registered, but not yet tested for reprotoxic effects, will have to undergo the Extended One-Generation Reproductive Toxicity Study if they are manufactured or imported in quantities over 1000 tonnes per year, which is the case of biphenyl.

Additionally, the information on reproductive toxicity is relevant for the PBT assessment considering that the classification as toxic to reproduction has a direct link to the T criterion (REACH Regulation, Annex XIII).

On the basis of available data, provided by Registrant(s) and found in the literature, no clear conclusions about the reproductive toxicity potential of the registered substance, biphenyl, can be made.

In the registration dossier, the Registrant(s) provided two studies in order to address the effects on fertility. A fertility study in rats by oral route (1960) (no GLP), with pre-mating, mating and until weaning treatments with administered doses of 5000 or 10000 mg/kg is available. No effects on reproduction and weaning were observed at those dietary doses before mating and throughout gestation. This study was assigned Klimisch 3 by the Registrant(s).

In a three-generation dietary exposure study (1953) (equivalent to an extended OECD 422) (no GLP) assigned Klimisch 4 by the Registrant(s), rats were administered 100, 1000 or 10000 mg/kg of biphenyl. At the highest dose tested (estimated 887 mg/kg bw/day in males and 1006 mg/kg bw/day in females), poor fertility, decreased litter size and poorer growth of the young (decreased body weights) was observed, compared to the controls. Data on food consumption and breeding rat weight are not available. The estimated concentrations (887 mg/kg bw/day e 1006 mg/kg bw/day) were similar to the dose that caused maternal toxicity in a developmental toxicity study (1979) (see below).

Based on a weight of evidence approach using the information from these two studies as well as supporting information from the repeated dose toxicity and carcinogenicity studies the Registrant(s) concluded in the registration dossier that there is no clear evidence that biphenyl would act as a reprotoxic agent.

However, for both fertility studies limited information on experimental design is available and a small number of animals were tested. The available information included deficiencies in procedure and uncertainties which preclude to conclude on fertility effects of biphenyl.

Concerning developmental toxicity, two studies are available in the registration dossier. As a key study (1979), assigned Klimisch score 2, by the Registrant(s), a test similar to OECD

Guideline 414 - Prenatal Developmental Toxicity Study (no GLP) by oral route/gavage is available. It presents incomplete or no information on environmental conditions and animals. Rats were administered 0, 125, 250, 500 or 1000 mg/kg bw/day biphenyl on gestation days 6–15. The maximum dosing volume exceeded recommended volume and there is limited information on clinical observations. Foetal toxicity, including non significant increases in foetuses with missing or non-ossified sternebrae and maternal toxicity at 1000 mg/kg bw/day were referred in the registration dossier. Considering the statistically significant increasing trend of missing and unossified sternebrae with dose and that this anomaly was more severe than the other anomalies, US EPA (2013) estimated a LOAEL of 500 mg/kg-day for increased incidence of foetuses with missing and unossified sternebrae and a NOAEL of 250 mg/kg-day, based on data from the publication (1979).

A summary of a mouse study (1988) according to EPA OPP 83-3 - Prenatal Developmental Toxicity Study (GLP) is included in the registration dossier. The study was assigned Klimisch 4 by the Registrant(s) since a full report is not available. Mice were administered 0, 125, 250, 500 or 1000 mg/kg bw/d. There was a high incidence of non-pregnancy in all groups. Food consumption was similar in exposed animals and control, maternal mortality and reduction in maternal weight gain was increased at the high-dose level (1000 mg/kg bw/day). Regarding litter effects, the total resorptions were significantly increased and mean litter size reduced at 1000 mg/kg bw/day. In this study it was concluded that biphenyl is foetotoxic and maternally toxic at 1000 mg/kg bw/day causing mortality of both dams and early-pregnancy loss including complete resorptions. The 500 mg/kg bw/day dose level was statistically a NOAEL for both dams and foetuses. The incidence of malformations was not increased.

It is considered that reasonable doubts were raised based on the anomalies observed in the referred fertility and developmental studies. Additionally, negative results observed in the studies similar to extended OECD 422 test do not indicate absolute safety with respect to uncertainties on reproduction.

Therefore, it is considered that no clear conclusion can be made on fertility or developmental toxicity effects from dietary exposure to biphenyl, justifying the request for further studies in the scope of substance evaluation. Consequently, there is an information gap for reproductive toxicity in Annex X and an Extended One-Generation Reproductive Toxicity Study (EOGRTS) should be performed.

Test design

Premating exposure duration

In order to fully address effects on fertility, especially on functional fertility and reproductive performance, the Extended One-Generation Reproductive Toxicity study shall be conducted using a premating exposure duration of 10 weeks. This should cover spermatogenesis and folliculogenesis before mating and assure adequately long exposure duration to reveal effects on reproduction in relation to general systemic toxicity from mating onwards.

According to the ECHA Guidance (Guidance on information requirements and chemical safety assessment, Chapter R.7a: Endpoint specific guidance, Version 4.0, July 2015) shorter exposure duration for parental P animals may be considered if animals of cohort 1B are mated to produce the F2 generation.

Extension of Cohort 1B:

It is considered that the uses covered by the updated registration dossier (by 28 June 2014)

do not lead to significant exposure of professionals nor consumers, therefore, the extension of Cohort 1B to produce the F2 generation is currently not required. However there are indications of a relevant mode of action related to endocrine disruption.

The concern for a mode of action related to endocrine disruption is based on the study by Petit et al (1997) in which a competitive bioassay was performed to determine direct interaction between rtER and xenobiotics. The estrogenic potency of xenobiotics was observed when the yeast cells and trout hepatocytes aggregated cultures were treated. Biphenyl and its metabolites (hydroxylated compounds) showed a dose-dependent induction of lacZ gene.

Biphenyl showed less estrogenic activity than the evaluated metabolites (2OH-Biphenyl; 2,2´OH-Biphenyl; 3OH-Biphenyl;4OH-Biphenyl and 4,4ÓH-Biphenyl) which exhibited clear estrogenic activity in the two bioassays. The results suggest that biphenyl is more than four orders of magnitude less active than 17β-Estradiol, which represents the maximum induction (reference unit). The maximal activity obtained with biphenyl metabolites is three orders of magnitude lower than 17β-Estradiol. In this study, biphenyl metabolites 3-Hydroxybiphenyl, 4-hydroxybiphenyl, 4,4*-dihydroxybiphenyl, showed a relative binding affinity for ER 1700-to 6700-fold lower than E2 but in the same range that several Aroclors and nonylphenol diethoxylate.

Supporting evidence is available from the USEPA report (2013), mentioned by the Registrants, that also includes indications of mode of action related to endocrine disruption activity of biphenyl and its metabolites. This report concludes that compounds without an hydroxyl group (e.g. biphenyl) were inactive estrogenically but estrogenic activity of the hydroxylated metabolites (4-hydroxybiphenyl, 3-hydroxybipehnyl, 2-dydroxybiphenyl, 4-4´dihydroxybiphenyl) is in the same order of magnitude than Bisphenol-A regarding relative gene activation of the Lac-Z gen of *Saccharomyces cerevisiae* based and estrogenic activity in MCF-7 cells and rat liver microsomes.

Thus, there are indications of one relevant mode of action related to endocrine disruption namely that biphenyl in available *in vitro* studies expressed estrogenic activity *via* its main metabolites. There is not sufficient information to conclude at which levels these oestrogenic phenolic metabolites occur in vivo.

One of the conditions for inclusion of the extension of Cohort 1B is not fulfilled, based on the updated dossier (by 28 June 2014) because there is no significant exposure to the consumers and/or professionals. However the condition of indication of one or more modes of action in relation to endocrine disruption is fulfilled. Thus, the Registrant(s) should expand the study design to include the extension of cohort 1B to produce the F2 if, before the time of the testing, new information on uses leading to significant exposure of professionals or consumers[2] according to criteria column 2 section 8.7.3 of Annex X is known to the Registrant(s). The justification for the extension must be included.

DNT cohort

Scientific articles reported effects on workers that showed evidence of neurotoxicity when exposed to concentrations of biphenyl exceeding the exposure threshold limits.

Several papers from Häkkinen and colleagues assessed the effects of the exposure of paper mill workers to biphenyl during the production of biphenyl-impregnated paper used to wrap

[2] As outlined in the REACH Guidance Document (Guidance on information requirements and chemical safety assessment, Chapter R.7a: Endpoint specific guidance, Version 4.0, July 2015) significant exposure can be expected "If the substance is intended to be used in the EU by consumers (i.e. members of the public) or professionals, either neat or in a chemical mixture and there is one very wide use or several limited uses potentially affecting many consumers and/or professionals, then this is considered as meeting the criterion."

citrus fruits. A total of thirty-three workers (32 men and 1 woman) exposed to biphenyl were included in a study (Seppäläinen and Häkkinen, 1975). Common complaints among these workers included fatigue, headache, gastrointestinal discomfort, numbness and aching of the limbs. Twenty-four of the 33 workers were subjected to neurophysiological examinations, including EEG and electroneuromyography (ENMG, consisting of nerve conduction velocity and EMG tests). Seppäläinen and Häkkinen (1975) noted that subjects often exhibited signs of dysfunction in both the peripheral nervous system, as evidenced by abnormal electroneuromyography (ENMG), and the central nervous system, as evidenced by abnormal electroencephalography (EEG) and abnormal distribution of alpha activity.

The authors concluded that "biphenyl can attack the human nervous system at several levels. The sites of greatest vulnerability are the brain and peripheral nerves". Additionally the authors noted that although the damage noted in the study was minor in degree the signs were persistent. Although there are limitations in this study the findings raise the concern on potential neurotoxicological effects which need to be clarified.

Another study (Wastensson et al., 2006) examined the prevalence and incidence of Parkinson's disease among workers at a facility manufacturing biphenyl-impregnated paper in Sweden. The study, in which medical records from workers that had worked in that facility anytime from 1954 to 1970 were analysed, was prompted by the recognition that three cases seen at a neurological clinic shared a history of work at this workplace. These authors identified 5 prevalent cases of Parkinson's disease among the 255 workers still alive compared with 0.9 cases expected for a group of that size, for a relative risk (RR) of 5.6 (95% confidence interval 1.9–13). Additionally the mean age at onset of symptoms (51 years) was lower than the mean at the Swedish population (66 years). Four of the patients had worked in the rewinding or drying section and the other operated the rewinder in another zone. As there were no biphenyl exposure levels available, it was estimated that the level of biphenyl in air would be more than two times higher than the existing TLV of 1.3 mg/m^3, based on a similar plant. These observations raise concerns but do not allow to conclude on the causal link between biphenyl and Parkinson's disease.

In their comments Registrant(s) state that the studies cited in the initial draft decision for inclusion of DNT Cohort refer to obsolete use of biphenyl impregnation into fruit wrapping tissue and those high exposures are no longer relevant for biphenyl and that the exposure to chemicals and job function were often poorly recorded and it is difficult to trace effects back to any single substance.

Although these studies refer to a former use in which high exposures occurred it is considered that the concerns regarding potential developmental neurotoxicity effects should be clarified.

The publication by Weeks & Lentle (1970) was mentioned by the Registrant(s) in their comments to the initial draft decision but was not included in the revised registration dossier. This publication refers to the use of organic reactor coolants and exposure to terphenyls mixtures. The potential hazards of the coolants considered in the planning of this study were: acute (damage to lung, skin and eyes) and chronic (damage to kidney, liver and blood forming organs; carcinogenic effects and induction of metabolic disorders). The authors stated that after the four years of operating experience with an organic cooled reactor the clinical material related to acute effects is less than required for adequate clinical evaluation.

The conclusion of this study states "The results of this investigation show that, within the parameters which were considered to be relevant, no harmful effects could be demonstrated." Registrant(s) commented that the study did not show any adverse health

effects in the worker Cohort from the exposure. However, considering that in these investigations no parameters on neurotoxicity were evaluated it cannot be concluded that there are no effects of neurotoxicity of biphenyl from this study.

Moreover the Registrant(s) in their comments state that extensive data from animal studies indicate that the nervous system is not the primary target of toxicity from ingested biphenyl, but kidney, urinary bladder and liver were affected in repeated dose studies.

The studies conducted under OECD test guideline 453 - Combined Chronic Toxicity\Carcinogenicity Studies, may include specific observations for clinical signs and histopathological examination of neurotoxicity and immunotoxicity. However the repeated dose animal studies reported in the dossier did not include examination of neurotoxicity parameters.

The 2-year oral bioassays in rats and mice (2002, 2005) included in the dossier (Repeated dose toxicity: oral) performed according to this guideline do not mention nervous system effects. No confirmation on this specific concern, nor information on the test procedure was included in the RSS and therefore the absence of results on neurotoxicity and immunotoxicity cannot be interpreted as lack of potential effects.

Although the evidence linking biphenyl to the nervous system effects in workers has some limitations and the uses of concern have been abandoned or risk management measures have been implemented to minimize the exposure of workers, it is considered that the information available raises concerns on neurotoxic effects. Therefore the potential neurotoxicity effects of biphenyl in reproductive toxicity need to be clarified. In addition, the estrogenic mode of action has been reported to be associated with developmental neurotoxicity (Ferguson et al. 2000; Frye et al, 2012) and there is indication for estrogenic mode of action. The estrogenic mode of action supports the request for the inclusion of DNT cohorts in the test design.

DIT cohort

It should be noted that in an oral repeated dose toxicity study in rats (2002) clear changes in the kidneys of male and female rats, prominent irregular scarring, lymphocytic infiltration, tubular atrophy and patchy tubular dilation to the point of cyst formation were registered in histopathological examinations. Calculi were present in the renal pelvis and similar deposits were observed in the kidney. Decreasing haemoglobin values were also observed, but were attributed by the authors to decreased food intake.

In their comments the Registrant(s) claimed that none of the arguments presented in the initial draft decision to include DIT Cohort constitute valid triggers. The adverse immune effects need to be non-stress-related, primary effects on the immune system. The effects described are immune responses secondary to general toxicity within the target organ, kidney, from physical presence of biphenyl-induced calculi and from the inflammatory infiltration seen in rat kidneys given high biphenyl doses. These calculi also caused physical damage to the organ as indicated by presence of blood in the urine during the studies.

Regarding the Registrant(s) comment it is considered that some of the effects of the repeated dose toxicity study in rats can be immune responses secondary to general toxicity within the target organ, kidney. The potential immune effects can be difficult to detect due to the mechanical damage of the kidney that can mask a primary immune response to the substance.

The US EPA (2013) toxicological review of biphenyl referred the results from the publications of Häkkinen et al. (1973; 1971) in which the key features at autopsy of a deceased worker included severe necrosis of most liver cells, unspecified changes in kidneys, degeneration of the heart muscles and also hyperactive bone marrow, and edematous changes in the brain.

Additionally it should be noted that immune responses as white blood cells and serum globulin levels were not reported in the studies included

There are indications that biphenyl shows estrogenic activity via its main metabolites. Estrogenicity mode of action has a critical role in the development and maintenance of immunity (Adori et al, 2010), therefore the indication from estrogenic mode of action for biphenyl and relevant metabolites supports the request for the inclusion of DIT cohort in the test design.

Additional comments from the Registrant

In their comments on proposals for amendment, the Registrant(s) argued that the results in Petit et al, 1997 for biphenyl and its metabolites indicate very weak if any in vitro activity. Moreover, there are 24 HTS activity assays for endocrine disruptors in ToxCast and it is clear that biphenyl does not activate large majority of them. Additionally it was referred by the Registrant(s) that biphenyl was active in two endocrine assays at high concentrations, approaching the limit test dose and was not active in thyroid and androgen assays. The Registrant(s) concluded that such sporadic responses in few of the assays does not equate to endocrine mode of action for biphenyl.

In response to these comments and as referred above ECHA refers to section "Extension of cohort 1B" where the indications for endocrine disruption mode of action are described.

The Registrant(s) in their comments also pointed out that the EOGRTS test guideline includes numerous endpoints in the P1 and Cohort 1A animals that will provide neurotoxicity and immunotoxicity data to satisfy the concerns expressed in the initial draft decision and that EOGRTS provides ample opportunity to confirm the absence of a primary neurotoxicity and immunotoxicity or DIT and DNT related effects from biphenyl even without the inclusion of Cohorts 2 and 3. Based on the scientific evidence presented, the Registrant(s) asked to remove the DIT Cohort and the DNT Cohort requirement from the EOGRTS for biphenyl.

In response to these comments, and regarding the possibility to confirm neurotoxicity and immunotoxicity in the P1 and Cohort 1A animals without the inclusion of DNT and DIT cohorts in the study design, ECHA considers that if these concerns arise from the assessment of the results of Cohort 1A it is not possible to include the DNT and DIT cohorts in the initial test design. More importantly, the concern triggering the need for DNT and DIT cohorts is already established as explained in this decision.

In their comments on proposals for amendment, the Registrant(s) agreed with the proposal from one MSCA to remove the DIT and DNT cohorts from the EOGRTS test design.

However it is considered that the referred studies raises concerns and therefore the potential developmental neurotoxicity and immunotoxicity effects of biphenyl need to be clarified.

The Registrant(s) in their comments requested that the timeframe for dossier update be extended to 36 months from the time of the Final Decision as the timeframe of 27 months

established in the initial draft decision was considered not achievable.

The Registrant(s) referred that EOGRTS is a relatively new and complex study design to examine multiple toxicity endpoints in a single study and that these studies have taken up to 24 months for completion from the time the protocol was signed to the finalization of the report; this did not include protocol design, development of analytical methods and scheduling of laboratory time. In addition, the necessary probe study preceding the EOGRTS includes milk transfer analysis and probing of non-linear kinetics to set dose levels has a cycle time of 6 months.

Considering that a probe study preceding the OECD 443 test to set dose levels, milk transfer analysis, probing of non-linear kinetics is not compulsory according to the OECD 443 test guideline but taking into account the remaining comments of Registrant(s) the deadline to provide the requested information is extended to 30 months.

Conclusion

Pursuant to Article 46(1) of the REACH Regulation the Registrant(s) shall submit the following information regarding the registered substance subject to the present decision: Extended one-generation reproductive toxicity study (EOGRTS) in rats, oral route (test method: OECD 443 including the Developmental Neurotoxicity (DNT) Cohort (Cohort 2A and 2B) and the Immunotoxicity (DIT) Cohort (Cohort 3).

One of the conditions for inclusion of the extension of Cohort 1B is not fulfilled. However, the condition of indication of one or more modes of action in relation to endocrine disruption is fulfilled. Thus, the Registrant(s) should expand the study design to include the extension of cohort 1B to produce the F2 if, before the time of the testing, new information on uses leading to significant exposure of professionals or consumers according to criteria column 2 section 8.7.3 of annex X is known to the Registrant(s). The justification for the extension must be included.

IV. Adequate identification of the composition of the tested material

In relation to the required experimental studies, 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 tests 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 tests must be shared by the Registrant(s).

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

In relation to the experimental studies 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 Registrant(s) to perform the studies 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://echa.europa.eu/regulations/appeals. The notice of appeal will be deemed to be filed only when the appeal fee has been paid.

Authorised[3] by Leena Ylä-Mononen, Director of Evaluation

Annex I: 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 II: References

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

Annex II:
References

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