EUROPEAN CHEMICALS AGENCY

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

For Triclosan, CAS No 3380-34-5 (EC No 222-182-2), registration number: [redacted]

Addressee: [redacted], registrant of Triclosan (Registrant(s)) [1]
Triclosan

This decision is addressed to all Registrants 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.

Registrants meeting the following criteria are *not* addressees of this decision:
i) Registrants who registered the above substance exclusively as an on-site isolated intermediate under strictly controlled conditions, and ii) Registrants 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 the European Chemicals Agency (ECHA).

Based on an evaluation by Bureau REACH on behalf of the Dutch Ministry of Infrastructure and the Environment (evaluating Member State Competent Authority, eMSCA) in cooperation with the Danish Environmental Protection Agency (the Competent Authority of Denmark), 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 does not take into account any updates of the registration of the Registrant(s) after 23 April 2013.

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 the Netherlands has initiated substance evaluation for Triclosan, CAS No 3380-34-5 (EC No 222-182-2) based on a registration dossier submitted by the Registrant(s) 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 for concern relating to its persistence, bioaccumulation and toxicity (PBT) properties and

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

endocrine disrupting (ED) properties, Triclosan was included in the Community rolling action plan (CoRAP) for substance evaluation pursuant to Article 44(2) of the REACH Regulation to be evaluated in 2012. The updated CoRAP was published on the ECHA website on 29 February 2012. The Competent Authority of the Netherlands was appointed to carry out the evaluation, in cooperation with the Competent Authority of Denmark.

The eMSCA 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 28 February 2013.

On 20 March 2013, 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.

The Registrant(s) provided comments to ECHA on the draft decision by the deadline of 23 April 2013.

On 25 April 2013, ECHA notified the eMSCA of the comments received. The eMSCA considered the comments received from the Registrant(s). The information contained therein was reflected in Information Required (section II) and the Statement of Reasons (section III). In accordance with Article 52(1) of the REACH Regulation, on 6 March 2014 the eMSCA 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.

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

On 10 April 2014, 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 the proposals for amendment within 30 days of the receipt of the notification.

The eMSCA has reviewed the proposals for amendment and amended the draft decision accordingly.

On 22 April 2014, ECHA referred the draft decision to the Member State Committee.

By 12 May 2014, the Registrant(s) provided comments on the proposed amendments and on the amended draft decision. The Member State Committee took into account the comments the Registrant(s) made on the proposals for amendment. It is to be noted that the Member State Committee only considered the Registrant(s)' comments that were related to the proposals for amendment (PfAs).

After discussion in the Member State Committee meeting on 10-13 June 2014, a unanimous agreement of the Member State Committee on the draft decision as modified at the meeting was reached on 12 June 2014. ECHA took the decision pursuant to Article 51(6) of the REACH Regulation.

II. Information required

II.I Concerns on persistency, bioaccumulation and toxicity (PBT)

Pursuant to Article 46(1) of the REACH Regulation the Registrant(s) shall submit the

following information using the indicated test method(s) and the registered substance subject to the present decision:

1. Simulation testing of Triclosan on ultimate degradation in fresh surface water (lake or river) and sea water performed as pelagic test (i.e. water only without addition of suspended solids) at an environmentally relevant temperature of at most 12 degrees centigrade as specified in Section III.I (test method: EU C.25/OECD TG 309).The test set-up shall enable to check the mass balance (using radiolabelled Triclosan) and the identification of transformation products relevant for PBT assessment (at a concentration of ≥ 0.1 % w/w unless it can be demonstrated that this is technically not possible).

II.II Concerns on endocrine disruption

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

2. Enhanced Developmental Neurotoxicity Study (test method: OECD TG 426 with relevant elements of Extended One-Generation Reproductive Toxicity Study, OECD TG 443).

The proposed study has the basis in the oral OECD TG 426 design, but shall additionally include several endpoints which are mandatory in the extended one generation study (OECD TG 443).Therefore, the OECD TG 426 study with oral gavage dosing during pregnancy and early lactation until direct dosing of the pups starts, shall also include measurements in the offspring of: anogenital distance, weight and histopathology of reproductive organs and include a quantitative evaluation of primordial and small growing follicles, as well as corpora lutea, estrous cyclicity and semen quality, as detailed in the OECD TG 443. Furthermore direct dosing of the pups is requested and offspring shall be exposed directly during the entire postnatal period, i.e. from the 3rd or 4th day after birth until weaning. When using direct dosing of pups, it may in the beginning of the dosing period for practical reasons be necessary to use a micropipette for administering Triclosan solution into the mouth of the offspring. Exposure shall continue using oral gavage when the size of the pups allows for this procedure to be used.

The study shall furthermore be performed in Wistar rats and the dose levels for the dams shall be 30, 100 and 300 mg/kg bw/day. For the direct dosing of the offspring the dose level shall be 15, 50 and 150 mg/kg bw/day using a dosing procedure and a dosing solution volume in e.g. corn oil, in accordance with paragraph 30 of OECD TG 443 and with a dosing volume as low as possible, in accordance with good animal welfare practice. If the Registrant(s) need to perform a range-finding study and results from such a study should indicate the need for modifying the proposed dose levels, this may be acceptable provided that robust scientific justification is given.

The study shall also include additional measurements of Thyroxine (T4) in all the dams during gestation, preferably around gestation day (GD) 15. All blood sampling shall be performed to minimize animal distress. A possibility is blood sampling from the tongue of the pregnant rats without anaesthesia, a method which has the advantage that potential animal loss because of use of anaesthesia is avoided. Should the performing laboratory however prefer to use another procedure for obtaining blood samples from pregnant dams, this shall be done in a way to minimize animal distress as mentioned above. 1-2 pups per litter shall be sacrificed for blood sampling and their trunk blood shall be used for the thyroid hormone measurements. The blood for measurements of T4 levels in the offspring shall be taken at a point in time when they are being directly dosed, preferable between prenatal development day (PND) 10 and 14. This sample shall be retrieved to make sure that the offspring have been exposed enough to Triclosan in the postnatal period to lower

their T4 levels, which may cause behavioral effects later in life.

3. Fish Sexual Development Test (FSDT, test method: OECD TG 234) with zebrafish or Japanese medaka.

The nominal test (target) concentrations shall, based on available existing information, be set at exposure concentrations of 15, 50 and 150 µg/l in a flow through system unless scientific evidence indicates that other exposure concentrations are more appropriate. The actual exposure concentrations shall be verified analytically as specified in the test guideline.

The study shall include the optional endpoints of the test guideline, i.e. gonadal histopathology (evaluation and staging of oocytes and spermatogenetic cells) to be able to characterize and better address any effects seen on sperm counts. If medaka is used also the genetic sex and secondary sex characteristics should be reported.

Pursuant to Article 46(1) of the REACH Regulation the Registrant(s) shall submit full study reports and robust study summaries for the information required under points 1-3 of Sections II.I and II.II.

II.III Concerns regarding cardiotoxicity

4. Available information on the effects of Triclosan on the cardiovascular system, including information on blood pressure, heart rate, electrocardiogram and human vigilance, as further specified in Section III.III.

II.IV Concerns regarding exposure

5. Further information on the environmental emission scenario 'Wide dispersive indoor use of reactive substances in open systems' in Section 9.4 of the Chemical Safety Report, as further specified in Section III.IV.

Deadline for submitting the required information

Pursuant to Article 46(2) of the REACH Regulation, the Registrant(s) shall submit to ECHA by 26 September 2016 an update of the registration dossier containing the information required by this decision.

Pursuant to Article 22(1)(e) and Annex I, 0.5. of the REACH Regulation the Registrant(s) shall revise their chemical safety assessment taking into account the information requested in this decision.

III. Statement of reasons

III.I Concerns on persistency, bioaccumulation and toxicity (PBT)

Based on the evaluation of all relevant information submitted on Triclosan and other relevant and available information on both the registered substance and the degradation product methyl Triclosan, and taking into account the comments of the Registrant(s), proposals for amendment submitted by Member State Competent Authorities/ECHA and the deliberations of the Member State Committee ECHA concludes that further information is required in order to enable the eMSCA to complete the evaluation of whether the substance is persistent, bioaccumulative and toxic (PBT) or very persistent and very bioaccumulative (vPvB).

The data for Triclosan in the chemical safety report contain bioconcentration factors in fish higher than 2000 and chronic NOECs or EC10s for algae and invertebrates below 10 µg/L. As a result, the B and T criteria of Annex XIII of the REACH Regulation are met, although the CSR concludes that the substance is only potentially B and does not meet the T criterion. The assessment strategy on PBT therefore focuses first on persistence of the registered substance. Several studies on the transformation product methyl Triclosan were initially foreseen to be requested in the present decision but have been removed during the decision making:

- Degradation simulation testing for Methyl Triclosan (test method: EU C.25/OECD TG 309);
- Bioaccumulation testing for Methyl Triclosan (test method: OECD TG 305);
- Long-term toxicity on invertebrates for Methyl Triclosan ((test method: EU C.20/OECD TG 211);
- Fish, early-life stage toxicity test for Methyl Triclosan (test method: OECD TG 210).

Several proposals for amendment triggered a reconsideration of the PBT testing strategy. With the initial testing sequence as outlined first in the draft decision sent to the Registrant(s) on March 2013, the overall substance evaluation would have caused a disproportionate delay in the generation of data necessary to clarify the identified concerns. Because all testing with methyl Triclosan is conditional to the results of the simulation studies on the registered substane, and because the interpretation of the results of these simulation tests is complex and often not unequivocal, no methyl Triclosan testing is required at this stage. The response of the Registrant(s) on the proposals for amendment on methyl Triclosan testing however has been taken into account.

If the registered substance is considered not to be PBT or vPvB based on the Annex XIII criteria, the remaining PBT or vPvB concerns for relevant degradation products such as methyl Triclosan may be subsequently addressed at a later stage to conclude the PBT assessment (REACH Article 46(3)).

1. Simulation testing for Triclosan

Initial requirement

According to the PBT assessment strategy in REACH (guidance R11.1), concerns on P should generally be addressed first before proceeding with consecutively the B and T criteria.

The data in the CSR concerning degradation are considered equivocal. In the following section, the weight of evidence in the registration dossier is assessed and additional information from the open literature is evaluated to justify further testing.

Ready biodegradability studies as included in the dossier show very little mineralisation, while inherent biodegradability tests and aerobic sewage treatment simulation tests do show the potential for almost complete degradation. The result from the Zahn-Wellens test (OECD TG 302B) shows just over 70% mineralisation within 7 days, a threshold that could be considered as not meeting the P criterion ([redacted]).

The CSR includes also simulation tests in soil and in water/sediment systems. The reported DT50s for one of the soil tests are 2.5, 3.3, and 2.7 days in three soils at 20°C and 11 d in the first soil at 10°C (study according to OECD TG 307). However, the dissipation of Triclosan from these soils is not following simple first-order kinetics, but is biphasic. The initial fast decrease completely corresponds with a fast formation of bound residues. This formation is complete after two weeks but amounts up to 60-76% in the soils. The slow dissipation that follows has corresponding half-lives for the parent compound of 33, 16, and

9.4 days in three soils at 20°C and 132 d in the first soil at 10°C, the latter being higher than the P criterion of 120 days in soil ([redacted]).

For sediment/water (study according to OECD TG 308) a similar situation is observed, although the formation of bound residue is less prominent than for soil (32-33%). The reported half-lives for the whole system at 20°C are 41 days for the river and 58 days for the pond system. For the river system the dissipation is significantly biphasic with the half-life of the parent compound for the slow phase being 93 days ([redacted]).

In both soils and sediments, mineralization is low, with cumulative mineralized amounts of less than 30% in sediments after 104 days and less than 20% in soils after 124 days. In soils and pond sediment methyl Triclosan was the major metabolite, in river sediment an additional unidentified metabolite was formed. The amount of methyl Triclosan ranged from 4 to 17% in the soils and from 3 to 5% in the sediments at the completion of the tests. From these results the question arises whether the substance is fast degraded or the observed fast dissipation is merely due to the formation of bound residue, of which the exact nature is unclear. The extent to which bound residues are formed is dependent on the applied extraction technique as well. For example, soxhlet extraction and harsh extraction acidified with hydrochloric acid extracted radioactivity additionally to the cold extraction. A further observation is the strong effect of temperature on dissipation of Triclosan, most likely related to different rates of biodegradation, which is for soil directly linked to the rate of formation of methyl Triclosan.

From several studies in public literature, both field and laboratory, it appears that the way of introducing Triclosan in the soil as well as the extraction procedure are essential to the formation of bound residues. When Triclosan is introduced together with biosolids from a sewage treatment plant, dissipation half-lives are remarkably longer, sometimes amply exceeding the P criterion of 120 days (e.g. Lozano *et al.*, 2012; Lozano *et al.*, 2010; Langdon *et al.*, 2011; Langdon *et al.*, 2012; Waria *et al.*, 2011; Kwon *et al.*, 2010; Walters *et al.*, 2010; Butler *et al.*, 2012; Carr *et al.*, 2011a,b; Cha and Cupples, 2010). The route of environmentally relevant emission through biosolids is especially important for Triclosan and other pharmaceuticals and personal care products (PPCPs). Most of these PPCPs enter the environment directly in the water compartment via the effluent of the sewage water treatment plants or indirectly by applying biosolids from these sewage treatment plants to agricultural soils.

In addition to the differences in the formation of bound residues, field studies show less dissipation of Triclosan than laboratory studies, possibly related to the fact that most laboratory studies were performed at room temperatures or even higher. An indication for such effect is also observed in the simulation study, in which the fate of Triclosan in one soil was studied at both 10 and 20 °C, leading to a difference of a factor 4 in half-lives [redacted]. Next to the temperature also the moisture content of the soil seems to play a role in the degradation of Triclosan, with higher methylation (formation of methyl Triclosan) under arid conditions, even approaching the wilting point (Butler *et al.*, 2012; Carr *et al.*, 2011a,b; Cha and Cupples, 2010). Because methylation is generally attributed to a microbial mediated process and bacterial activity is higher in moist soils, this process is not well understood.

It remains unclear to what extent the laboratory degradation tests with freshly spiked Triclosan are representative of the fate of Triclosan under field conditions. The long half-lives observed under field conditions are confirmed by QSAR estimates (BIOWIN), which also result in limited degradation indicative of persistence.

Most of the studies in the registration dossier as well as in public literature have focussed on

the soil compartment. Surface water is an equally or even more important receiving compartment for Triclosan because of the direct emission of sewage treament plant effluents to surface water. This is confirmed by the widespread occurence of both Triclosan and methyl Triclosan in fresh surface water and sediments and seawater as reported in the public literature (e.g. Lindström *et al.*, 2002; Xie *et al.*, 2008). Biodegradation in water has not been considered in a degradation simulation study yet. A degradation simulation study in water has the advantage of the absence of bound residues, which will make interpretation of the results more straightforward.

Summary of Registrant(s)'s comments and responses to comments

In their comments on the Draft Decision, the Registrant(s) stated that a surface freshwater and seawater study (OECD TG 309) should not be performed. Various arguments were brought forward and are presented here, together with the corresponding reply. Note: OECD TG 308 and OECD TG 309 are interchanged in the comments by the Registrant(s). The correct terminology has been adopted in the decision.

a) The Registrant(s) do not agree that pelagic surface water is the most relevant compartment for Triclosan in the environment as shown by the physical-chemical properties and a water-sediment study submitted in the dossier (OECD TG 308). Triclosan rapidly dissipates from the water phase, which corresponds to an increase of Triclosan in the sediment phase and suspended particular matter. A pelagic study does not seem to be environmentally relevant as in real surface waters a solid phase in form of suspended particulate matter or sediment is always present. According to the Registrant(s), the results from the available water-sediment simulation study demonstrate that the dissipation times for water (< 2 d) and sediment (ea. 60 d) are clearly below the P/vP criteria.

   The eMSCA noted that the ratio of water and sediment in an OECD TG 308 test (3:1 to 4:1) corresponds to a very shallow water layer, which is completely different from the large water columns that are present in the environment. Consequently, results from the OECD TG 308 test cannot be used as a measure for the environmental distribution of a substance. The mass ratio of the substance in water and sediment in the environment is completely different from that in the test system in an OECD TG 308 study.

   This is confirmed by modelling data, e.g. by the program EPI Suite from the US Environmental Protection Agency. With the default calculation of EPI Suite (emission 100% to water, K_{oc} value based on molecular connectivity indices (MCI) with all other values taken as defaults, with very long half-lives in water, sediment and soil) it appears that the mass amount in water is at least half that in sediment (36% versus 64%). If the half-lives of Triclosan are set to those of the P-criteria under REACH, the mass amount in water is almost twice that in sediment (62.1% versus 37.9%). The value used for the partition coefficient between organic carbon and water is even more important. If the K_{oc} value based on log K_{ow} is used, 61.2% or 81.3% of Triclosan will be present in water, dependent on the half-lives estimated either by EPIWIN or half-lives set to the REACH criteria. Both values are still relatively high in comparison to the value for log K_{oc} of 2.92 that is used in the dossier. With this value for log K_{oc}, dependent on the half-lives used, an estimated 94.5% or 97.2% of Triclosan will be in the water phase. These calculation thus clearly show that a significant part, if not the majority of Triclosan will reside in surface water in the environment, showing that freshwater is a relevant compartment for Triclosan.

   The half-life of Triclosan in water from a water-sediment study (OECD TG 308) only

reflects dissipation due to fast sorption to the sediment in such a system. It cannot be used to compare with the P/vP criteria for surface water that are clearly defined for degradation (Commission Regulation (EU) No 253/2011) and not for dissipation. Consequently, the degradation in an OECD TG 308 test is not a measure of degradation in the pelagic water column, but of degradation in the sediment instead, often reflecting anaerobic degradation.

Besides that, the interpretation of the available OECD TG 308 study is hampered by the fact that bound residue is formed to a high degree. The obtained half-lives therefore reflect dissipation rather than degradation. This effect is also observed for soil, for which a large discrepancy in half-lives exists between field studies or studies with Triclosan incorporated in activated sludge on the one hand and soil freshly spiked with Triclosan in the available OECD TG 307 study on the other hand.

The pelagic test (OECD TG 309) uses natural water, which includes suspended particular matter that is naturally present in the water column. Any effects of partitioning between this suspended particular matter and the dissolved phase on the fate of Triclosan will thus be dealt with in this type of simulation test. Hence, a simulation test in freshwater (OECD TG 309) is required.

b) The Registrant(s) agree that the sewage treatment plant effluent is the most important route of Triclosan input into the environment but does not agree that pelagic surface water is the most relevant compartment for Triclosan in the environment as shown by various sewage treatment plants monitoring data. Based on these data the Registrant(s) conclude that Triclosan is removed from wastewater to a large extent (usually > 90 %) not only by adsorption but also by mineralization (e.g. Bester 2005, Heidler *et al.*, 2009, Kuch *et al.*, 2003).

The eMSCA accepted that a large fraction of Triclosan is removed in the sewage treatment plant (STP). Despite this, STPs do not remove all Triclosan and as a result, STPs are the most important source for emission of Triclosan (and of methyl Triclosan) to the aquatic environment. Due to its wide dispersive use, monitoring studies report Triclosan in relatively high concentrations in effluents of STPs and in receiving surface waters, where degradation is suspected to be much lower: Triclosan appears to be a ubiquitous substance in fresh surface waters all over the world. Triclosan occurs in many fresh surface waters, including large water bodies such as the Great Lakes in North America (Blair *et al.*, 2013; Andresen *et al.*, 2007), large Swiss lakes (Lindström *et al.*, 2002; Singer *et al.*, 2002, Tixier *et al.*, 2002), and European rivers (Bester, 2005; Van Wezel & Jager, 2002), with typical concentrations of one to tens of nanograms per liter. This indicates that pelagic surface water is a highly relevant compartment for Triclosan in the environment.

c) The Registrant(s) do not agree to carry out an OECD TG 309 in pelagic marine water, as he does not agree that pelagic marine water is a relevant compartment for Triclosan, as shown by the physical-chemical properties, a water sediment study (OECD TG 308) and various sewage treatment plants monitoring data. He argues that, since extensive elimination of Triclosan occurs in STPs and along the river sections, measured values in the marine compartment are generally extremely low or Triclosan has not been measured at all in the marine area. In one publication where coastal water from a Swedish area with heavy industry and chemical manufacturing was analysed, Triclosan was detected at 60 ng/L (Remberger *et al.*, 2002; cited in Danish Environmental Protection Agency, 2003). Further data exist for Norway, where marine waters adjoining municipal and hospital sewage treatment plant outflows were investigated (Weigel *et al.*, 2004) and Triclosan was not detected (LOQ = 0.24 ng/L).

Overall, the concentrations in the marine system can be expected to be low regarding the removal efficiency of European sewage treatment plants, the low Triclosan concentrations of sewage treatment plant effluents and the fact that direct discharge of raw sewage is restricted within the European Union. The Registrant(s) argue that apart from the low concentrations of Triclosan in marine water, a pelagic test system is not environmentally relevant for Triclosan based on its relatively high adsorption potential and the overall presences of a solid phase (suspended particulate matter and sediment) in natural marine systems.

The Registrant(s) observe that Triclosan is removed to a large extent from wastewater in sewage treatment plants. Nevertheless, Triclosan is released via the effluents of sewage treatment plants into rivers and other receiving water bodies at the ng-scale. The Triclosan concentrations show a clear decline starting from the point of discharge down along the river. In a publication by Sabaliunas *et al.* (2003), the Triclosan concentration at 20 m below the sewage treatment plant discharge point was determined to be 80 ng/L. This value decreased with increasing distance. At 750 m downstream the Triclosan level was 53 ng/L; at 1500 m 43 ng/L and at 3500 m 44 ng/L were detected.

The eMSCA observed that Triclosan has been detected in numerous marine waters around the world, including the German Bight (Xie *et al.*, 2008), Victoria Harbour in Hong Kong (Chau *et al.*, 2008), Charleston (SC, USA) Harbor and estuary (DeLorenzo *et al.*, 2008; Fair et al, 2009; Hedgespeth *et al.*, 2012), Southern California Bight (CA, USA) (Vidal-Dorsch *et al.*, 2012; Bay *et al.*, 2012), coastal waters around Singapore (Bayen *et al.*, 2013), Greenwich Bay (RI, USA) (Katz *et al.*, 2013), Lower Hudson estuary (Wilson *et al.*, 2009), and mangroves in India (Ramaswamy *et al.*, 2011). In these coastal areas concentrations have been reported that are sometimes similar to fresh surface water, e.g. concentrations in the German Bight (Xie *et al.*, 2008) are actually comparable to the concentrations measured in the river Ruhr (Bester, 2005). The concentrations of Triclosan in a large part of the German Bight, which represent a very large water body, do still exceed the value of 1 ng/L and values up to 7 ng/L were found (Xie *et al.*, 2008). Other marine data show concentrations comparable to those in the German Bight and some studies have also shown that even higher concentrations occur in coastal areas (e.g. Hong Kong, Singapore, India). It can be concluded that Triclosan also reaches the coastal areas.
For the dissipation of Triclosan in rivers, the Registrant(s) refer to a study (Sabaliunas *et al.*, 2003) which concerns the emission of Triclosan from an STP to a shallow brook with a depth of 0.5 m, determined in early September. Except from the pH (which was only around 7), these seem to be rather favourable conditions for photolysis. It can be concluded from monitoring that this study cannot be considered as representative of the overall persistence of Triclosan.

As illustrated above concentrations in the marine environment are still relatively high. The fact that water is an important compartment for the total hold-up of Triclosan in the environment is shown not only by the monitoring data, as well as by fate modelling, which shows that a large part of the chemical ends up in water (see reply under item a). Further evidence for the fact that significant amounts of Triclosan reach the marine environment comes from the study in the Southern California Bight: Triclosan had the lowest dilution factor (concentration ratio) between the effluent of an STP and sea water of the seven investigated contaminants of emerging concern (Vidal-Dorsch *et al.*, 2012).

In conclusion, Triclosan is measured in coastal seawater in relatively high

concentrations. In most studies the water is filtered, thus without suspended particulate matter. If Triclosan is adsorbed to suspended particulate matter to a significant extent, total concentrations are even higher. Further, the OECD TG 309 pelagic test contains the naturally present suspended particulate matter. Because marine water bodies are very large and the water column deep enough to limit the role of photolysis to a minimum, Triclosan is suspected to be persistent in seawater. Hence, apart from a simulation test with freshwater, a simulation test with marine water (OECD TG 309) shall be performed as well.

d) The Registrant(s) observe that Triclosan is also degraded by abiotic processes that have been shown to be relevant under environmental conditions as well (e.g. rapid phototransformation in water with a half-life of<< 2 d, Tixier *et al.*, 2002).

The eMSCA acknowledged that photolysis of Triclosan can be very fast, under specific conditions. First, only with sufficient sunlight photolysis occurs, which means that this process is restricted to the surface layer of the water, due to the attenuation of UV-light in the water column. For example, for Lake Greifensee it was shown that at 50 cm depth the photolysis rate was already reduced to 5% of the rate at the surface (Tixier *et al.*, 2002). For several Swiss lakes (Zürich, Greifensee) it appears that the overall degradation half-life is much longer (60 days or more) than what is observed in the epilimnion, based on estimated input of Triclosan from STPs and rivers, and Triclosan removal by river outflow in combination with the lake volume and observed concentrations (Lindström *et al.*, 2002). The relatively low contribution of photolysis to the overall half-life of a substance is also shown by modelling, which shows relative fast degradation in water bodies up to 3 m, such as rivers, but virtually no degradation in deeper water layers such as lakes and coastal areas (Jiménez & Van de Meent, 2011).

Further, the overlap between the absorption spectrum and the solar spectrum is such that only the ionized form is photolysed, which means that under neutral and acidic conditions almost no photolysis takes place and the significance of photolysis is restricted to slightly alkaline water bodies (Tixier *et al.*, 2002).

Summary of proposals for amendment made by ECHA and MSCAs, Registrant(s)'s comments on them and response to Registrant(s)'s comments

ECHA and one MSCA made proposals for amendments questioning the simulation testing sequence for Triclosan and methyl Triclosan. ECHA suggested that the results of simulation testing on Triclosan might show that it fulfils the P criteria in Annex XIII and that no further testing would be necessary on methyl Triclosan in that case. ECHA proposed to give the Registrant(s) the possibility to test Triclosan and methyl Triclosan sequentially. One MSCA suggested to test only one source of freshwater for Triclosan which was the intention of the eMSCA. In addition, they suggested to test seawater only, due to the expected slower biodegradation in seawater than in fresh water. As an alternative, they suggested to test seawater first before proceeding to freshwater if still considered necessary to confirm the persistence properties of Triclosan.

One MSCA suggested to take any relevant transformation product into account in the PBT assessment, present at ≥0.1% w/w in the aquatic simulation tests. The eMSCA agreed to this suggestion.

In relation to the relevant temperature for the requested simulation test, ECHA suggested that 12 degrees centigrade would be a more appropriate temperature than the suggested 10 degrees centigrade. Twelve degrees is the value used in the REACH guidance for

freshwater, sediment and soil (Chapter R.16.6.4) and in the EUSES program, reflecting the agreed default environmental conditions in the EU.

In relation to the deadline in the decision to provide the requested information, ECHA noted that the current deadline appears too short to take all steps in the sequential testing on PBT properties into account. A similar PfA was received from one MSCA, suggesting that the time needed might even be seven years.

These proposals for amendment triggered a reconsideration of the PBT testing strategy. The eMSCA came to the conclusion that with the originally proposed testing sequence, the overall substance evaluation would suffer from a disproportionate delay. The eMSCA has therefore rephrased the decision to focus the PBT part on Triclosan only. This would allow for an evaluation of the PBT properties of Triclosan first within a reasonable amount of time. Because all testing with methyl Triclosan is conditional to the results from the simulation studies (endpoint 1), and because the interpretation of the results of these simulation tests is complex and often not unequivocal, all methyl Triclosan testing was deleted from section II.

It can not be predicted beforehand, whether freshwater or seawater would be the critical compartment for persistence: The half-life in seawater is expected to be longer than in freshwater, but the P criterion for seawater (60 days) is also longer than for freshwater (40 days). Only if the results cleary show that based on a single environmental medium, the substance is very persistent (vP), no further testing would be needed for the second medium. In addition, results of tests in both freshwater and seawater will provide a more robust basis for concluding on persistency of Triclosan based on a weight of evidence approach if needed.

Given the fact that both the test set-up (i.e. climate conditioning, vessels etc.) and the chemical analysis (same substance, same analytical protocol and apparature) are the same for the test in freshwater and seawater, the eMSCA considered it appropriate and proportionate to perform both tests simultaneously. This will avoid unnecessary costs and undue delay, especially because similar tests may have to be repeated if the parent proves not to be persistent and simulation testing may need to be performed for the metabolite methyl Triclosan.

By limiting the requested information to Triclosan only, the eMSCA did not see a need to change the ultimate timeframe of 24 months to complete the information required in this decision and update the registration dossier. This is also in accordance with the estimated time needed as indicated in the comments of the Registrant(s) on the PfAs (10 months for synthesis of radiolabelled Triclosan and 12 months for the surface water simulation tests in freshwater and seawater).

To facilitate a detailed review of the generated information, especially since possible requests for further studies on methyl Triclosan are conditional to the outcome of these studies, full study reports are required from the Registrant(s).

The Registrant(s) commented on these PfAs and repeated his objection to the relevance of the pelagic compartment. The Registrant(s) cite in addition to the studies mentioned in earlier comments two more studies ([redacted]; Procter & Gamble, 1992) to show that the removal in a sewage treatment plant is high and substantial mineralization occurs. It should be noted that the relevance of the fresh and marine water compartment for Triclosan and methyl Triclosan was not addressed in the received PfAs. The PfAs only addressed the number of simulation tests to be performed and the possibilities for sequential testing and the order of such testing.

The eMSCA was still of the opinion that the pelagic compartment is very relevant for Triclosan for the reasons described above in the response to the initial comments of the Registrant(s). Despite the high removal in sewage treatment plants, Triclosan is ubiquitous in the aquatic environment as shown by the large number of monitoring data for Triclosan in water, which are summarised above.

In his response to the PfAs, the Registrant(s) argued for the marine environment that the concentrations in the marine environment are generally extremely low. Further, it stated that the data for marine water in Asia are not representative for Europe. These issues address the relevance for the marine compartment, which was not addressed in the PfAs. In the response to the initial comments from the Registrant(s), the eMSCA has included several monitoring data for Triclosan in the marine environment. These monitoring data include data from other parts in the world, including Europe (German Bight). These data from the German Bight showed that the concentrations were comparable to those in German rivers (see above).

Despite the fact that the Registrant(s) contest the relevance of Triclosan in water, but especially in seawater, he agreed to perform the OECD TG 309 test only with seawater. The eMSCA was of the opinion that both tests with fresh water and seawater shall be performed simultaneously for the reasons described above in the response to the PfAs.

In response to the simulation testing, the Registrant(s) argued that the OECD test guidelines 307, 308, and 309 define major transformation products as those that have a concentration that is more than 10% of the initial applied test concentration. As such, it is suggested that the identification of the transformation products is not necessary according to the OECD test guidelines. However, the OECD test guidelines for water sediment systems (OECD TG 308) and surface water (OECD TG 309) state the following:
"In general, transformation products detected at ≥10% of the applied concentration at any sampling time should be identified unless reasonably justified otherwise. Transformation products for which concentrations are continuously increasing during the study should also be considered for identification, even if their concentrations do not exceed the limit given above, as this may indicate persistence."

From this it can be concluded that the trigger of 10% is certainly not a fixed value and that also the OECD test guidelines advise to identify persistent substances. In the cited sediment-water simulation study ([redacted]), such a continuous increase of methyl Triclosan was in fact observed for at least one of the two sediments. Quantification of any metabolite with a percentage lower than 10% of the applied concentration should be performed anyhow, because the OECD test guidelines state that the mass balance of total radioactivity should be within 90% and 110% of the initially applied dose of the radiolabelled substance. The Registrant(s) argue that analysis of transformation products at the threshold of 0.1% that is given in the REACH guidance for PBT assessment cannot be achieved without considerable effort, both for the limit of quantification and the limit of detection. However, if the percentage of transformation products is similar to those in the sediment-water study, the percentages of individual metabolites are up to 5%, which is almost a factor of 50 higher than this threshold value of 0.1%. The eMSCA therefore was of the opinion that a simulation test in water should be conducted with quantification of transformation products in a way that a full mass balance can be achieved. Further, those transformation products that appear to build up during the course of the test, should be identified, unless it can be demonstrated that this can not be achieved with reasonable efforts.

The Registrant(s) also indicated in his comments to the PfAs that he agrees with ECHA that

the eMSCA did not give a reason for the test temperature of 10°C for the simulation tests according to the OECD TG 309. The Registrant(s) state that the temperature for freshwater would be 12°C, and for seawater 9°C, according to the Technical Guidance Document (EU TGD – Part II; EC, 2003), while the temperature of 10°C is typical for simulation tests in soil. After the comments received from ECHA the temperature for both freshwater and seawater simulation tests was changed to 12°C. This is the value used in the REACH guidance for freshwater, sediment and soil (Chapter R.16.6.4) and in the EUSES program, reflecting the agreed default environmental conditions in the EU.

The Registrant(s) indicated that for practical reasons he would prefer to perform the simulation tests at 20°C and apply a temperature correction afterwards. The Registrant(s) mention that this is done in the biocides framework, but also described in the REACH guidance document R7.B. The temperature correction is however not unambiguous. Therefore, new simulation tests within the REACH framework are performed at environmentally relevant temperatures, which is 12°C for freshwater.

For Triclosan, a relative large difference in half-life between 20°C and 10°C was observed in the soil simulation study included in the registration dossier. The eMSCA was therefore of the opinion that the half-life should be directly determined at the environmentally relevant temperature, to prevent any debate on the interpretation of the half-life observed in the tests afterwards.

The Registrant(s) agreed with ECHA and one MSCA that the overall timeframe needed to complete all testing on Triclosan and methyl Triclosan would be much longer than indicated in the draft decision. The Registrant(s) also agreed with ECHA and one MSCA that the testing on methyl Triclosan should be dependent on the outcome of the simulation studies with Triclosan.

The eMSCA reconsidered and changed the information requested but did not change the timeframe.

Conclusion

Pursuant to Article 46(1) of the REACH Regulation, the Registrant(s) is required to carry out the following study using the registered substance subject to this decision: Simulation testing on ultimate degradation in surface water, in fresh surface water (lake or river) and sea water performed as pelagic test (i.e. water only without addition of suspended solids) at an environmentally relevant temperature of a maximum of 12 degrees centigrade (test method: EU C.25/OECD TG 309). This temperature was decided based on all comments referring to the relevant environmental temperature mentioned in the TGD (2003) and REACH guidance documents (Chapters R7, 11 and 16). This will allow the Registrant(s) to perform the freshwater and seawater simulation tests simultaneously. The test set-up should enable to check the mass balance (using radiolabelled Triclosan) and the identification of transformation products relevant for PBT assessment (at a concentration of ≥ 0.1 % w/w) unless it can be demonstrated that this is technically not possible.

III.II Concerns on Endocrine Disruption

Based on the evaluation of all relevant information submitted on Triclosan and other relevant and available information on the registered substance and taking into account the comments of the Registrant(s), proposals for amendment submitted by Member State Competent Authorities/ECHA and the deliberations of the Member State Committee, ECHA concludes that further information is required in order to enable the eMSCA to complete the evaluation of whether the substance is an endocrine disruptor or not.

2. Enhanced Developmental Neurotoxicity Study (OECD TG 426 enhanced with relevant elements of OECD TG 443)

2.1 Concerns for Endocrine disruption

2.1.1. Estrogenicity (E) and Androgenecity (A)

In vitro data (E+A)

Triclosan affects many test systems *in vitro*, including some which indicate potential endocrine disrupting activity. In a study by Gee *et al.* (2008) it was shown that Triclosan was capable of producing both estrogenic and androgenic effects, as it displaced radiolabeled estradiol from estrogen receptors (ER) of MCF7 human breast cancer cells, whilst also inhibiting testosterone from binding to the rat androgen receptor (AR). Antagonistic activity in both ER- and AR-responsive bioassays was also seen by Ahn *et al.* (2008), and Triclosan has been shown to bind to the AR in studies by Chen *et al.* (2007). Recently Christen *et al.* (2010) also found Triclosan to act as both a partial AR agonist and as an AR antagonist, whereas Svobodova *et al.* (2009) did not find any androgenic activity in the bioluminescent androgen assay nor any estrogenic effects in a β-galactosidase assay.
In vivo data, human health and mammalian wildlife (E+A)

In vivo, Triclosan has also been shown to have adverse effects on some estrogen sensitive reproductive endpoints. Stoker *et al.* (2010) performed both an uterotrophic and a pubertal assay in female Wistar rats, and the dose levels in both studies were 1.2, 2.4, 4.7, 9.4, 18.8, 37.5, 75, 150 mg Triclosan/kg/day. In the uterotrophic assay, immature rats were dosed for 3 days either with Triclosan alone or together with estradiol, whereas the females in the pubertal assay were dosed with Triclosan alone from PND 22-43. In the pubertal assay the highest dose caused advanced vaginal opening, increased uterine weight and altered estradiol levels, indicating estrogenic effects *in vivo*. Triclosan in itself did not affect uterine weight in the 3-day assay, however, doses of 4.7 mg Triclosan/kg/day and above potentiated the effects of estradiol.

In a study looking at reproductive effects of Triclosan in males, Kumar *et al* (2009), dosed adult male Wistar rats (n=8) with Triclosan for 60 days. The dose levels were 5, 10 and 20 mg/kg/day, and the dose of 20 mg Triclosan/kg/day caused decreased weights of several reproductive organs and histopathological changes in these, together with decreased levels of FSH, LH and testosterone. Furthermore decreased sperm production and altered gene expression of androgen regulated genes was seen. However, the Triclosan used in this study may have been contaminated by dioxins, which could account for the adverse effects seen on the male reproductive system.

No significant effects on timing of sexual maturation or reproductive organ weights were seen in a study by Zorilla *et al.* (2009), where young Wistar males were dosed daily with 3, 30, 100, 200, 300 mg/kg/day by gavage, from PND 23-58. Some developmental studies have also been performed. The Registrant(s) have performed a two-generation guideline study according to OECD TG 416 (earlier version from before the last update of the test guideline from 2001) in which Sprague-Dawley rats were dosed with Triclosan doses of 300, 1000 and 3000 ppm (approximately 30, 100 and 300 mg/kg/day). This exposure did not affect fertility or reproduction in the rats, however, because the study was from 1988 it had some major shortcomings with respect to assessing the endocrine disrupting potential of Triclosan, as neither weight nor histopathology of reproductive organs or semen quality was investigated (Morseth, 1988).

Recent developmental toxicity studies on Triclosan have also shown no treatment-related reproductive toxicity effects on gestation length, offspring viability, sex ratio, or pup body weights (Paul *et al.,* 2010b, 2012). Unfortunately these studies also did not investigate reproductive organ weights; histopathology or reproductive hormone levels in the offspring, because the focus of the studies was on the thyroid disrupting effect of Triclosan, and the possible mechanisms behind these effects.

When the available *in vivo* and *in vitro* data is included in the assessment of Triclosan's endocrine disrupting potential on the reproductive system, it becomes clear that Triclosan may cause adverse effects. Furthermore, it seems biologically plausible that the adverse effects observed *in vivo* could be caused by the endocrine modes of action found *in vitro*. However, since still only very few studies have investigated endocrine sensitive reproductive endpoints *in vivo*, and there are conflicting results, there is a need for further investigation of Triclosan's effects on reproduction and on endocrine sensitive endpoints.

Summary of the Registrant(s)'s comments and the responses to comments.

In their comments on Estrogenicity (E) and Androgenicity (A) / In vivo data human health and mammalian wildlife (E+A) / Relevance of published studies, the Registrant(s) were of the opinion that Triclosan used for in vitro and in vivo studies presented in the open literature could be contaminated, which could account for the observed effects on the reproductive system. The arguments brought forward by the Registrant(s) are presented here, together with the corresponding reply of the eMSCA.

a) The Registrant(s) comments on the quality of the test material. The Registrant(s) state that while it is true that some studies in the public domain point to endocrine activity of Triclosan in cell lines and reporter assays, the quality of the test material has often not been considered. From in vivo results, such as the study by Kumar *et al.*, 2009 who reported adverse reproductive effects in male rats, there is no reference to the analytical purity of the test material, other than the percentage purity of the Indian material used. In contrast to Kumar's findings, several studies in rats and other species conducted with USP grade material found no effect on the morphology of the testes or epididymis, which suggests that USP grade Triclosan does not cause these effects in rats.

   The eMSCA noted that the quality of the test material was probably not considered in a number of the performed toxicity studies. However, if Triclosan used for scientific testing was contaminated with dioxins, it appears likely that this Triclosan could also be used for other purposes, and therefore could be a source of human and environmental exposure. In the description of the Kumar *et al.* study in the DD, it was already stated that Triclosan used in this study may have been contaminated, why relatively little emphasis was put on these results. Since no actual results on weights or morphology of male reproductive organs was presented anywhere in IUCLID and since these endpoints were not investigated in the performed two-generation study, a proper evaluation on the effects of Triclosan on male reproductive disorders could not be performed.

b) The Registrant(s)'s comments on 'validity of the two generation study'. The Registrant(s) is of the opinion that in general information gained from a two-generation study can be regarded as minimal if a 90 day study has been performed, and that enough data is already available for Triclosan to conclude lack of effect on male fertility. Mangelsdorf *et al.* have concluded that histology and reproductive organ weights from repeated dose studies may be used to identify adverse effects on male reproduction and in fact be more sensitive than fertility parameters measured

during multi-generation studies. If a 28 day (or 90 day) study reveals neither significantly elevated testis or ovary weights nor histopathological alterations in those organs, the weight of evidence is that effects on reproduction are also not expected (Mangelsdorf *et al.*, 2003). Furthermore, a comparison of more than one hundred 90 day studies with two-generation studies (Janer *et al.*, 2007), showed that the NOAELs differed by less than the variation limit of studies, i.e. a factor of two. Therefore, the information gained from a two-generation study could be regarded as minimal, if a 90 day study had been performed. Since the effects on the weight and histopathology of the gonads of several species are absent in studies conducted with USP grade Triclosan, although the present OECD TG 416 requires fertility parameters (i.e. semen analysis) that were not assessed during the available two-generation study, the available data are sufficient to assess the endpoint 'male fertility'.

The eMSCA observed that while it is true that adverse effects in histopathology and reproductive organ weights from 90 day studies may be present at lower doses than those for example affecting fertility-index in a two-generation study, this does not mean that 90-day studies provide sufficient information to safely assess possible reproductive effects. The conclusion reached by Janer *et al.* (2007) in their retrospective analysis of added value of the two-generation study was that repeated dose studies may be used to identify adverse effects on male reproduction, because if clear indications of toxicity to reproductive organs were seen in a rat subchronic study, this was sufficient for classification for toxicity to fertility. If however no reproductive effects were seen in a subchronic study, this did not necessarily exonerate the substance for reproductive effects. The authors identified 13 studies, corresponding to 10 substances (out of 30 substances toxic to fertility) for which the subchronic study did not provide any indication of toxicity to fertility, whereas the two-generation study did. According to the authors, in some of the cases, a subchronic study might have shown indications of toxicity to fertility, if appropriately designed. However, even an 'ideally' designed subchronic study would probably not be able to identify all reproductive toxicants detected in the two-generation study. So while true that the two–generation study had little impact on changing the overall NOAEL available from a subchronic (or chronic) studies, it had a marked impact on classification for toxicity to fertility, as about one-third of the substances shown to be toxic to fertility in the two-generation study did not show any sign of that in the 90-day study.

Based on available data from the open literature, together with results supplied by the Registrant(s), the main concern with regard to Triclosan's possible effect on the reproductive axis seems to be its estrogenic properties (i.e. its ability to potentiate the effects of estradiol at very low doses (Stoker *et al.*, 2010; Louis *et al.*, 2013)). However, due to the studies by Kumar *et al.* (2009) a possibility of effects on male reproduction is also present. Moreover the key hamster study (1999) included in the registration dossier in accordance with OECD TG 451 (Carcinogenicity Studies,1981 version) indicated abnormal spermatogenic cells, absent or reduced numbers of spermatozoa, and partial depletion of one or more generations of germ cells in males treated with 250 mg/kg/bw of Triclosan. Finally recent published literature (Lan Z *et al.*, 2013) indicates that Triclosan exhibits a tendency to accumulate in the epididymis and may cause sperm toxicity in sprague-dawley rats. The available data from 28-days and 90-days studies are not sufficient to clarify suspicions of reproductive toxicity, because dosing in these studies is not performed during the sensitive windows of reproductive development. Furthermore no records of reproductive organ weights, histopathology or sperm counts from the performed two-generation study are present. This makes it difficult to properly assess effects of Triclosan on reproduction. Compared with the two-generation study (OECD TG 416), the new extended one-generation

study (OECD TG 443) is more suitable for identifying substances that act as reproductive toxicants including endocrine disrupters, as it requires an increased number of pups to be examined and includes an increased number of relevant reproductive toxicity endpoints (OECD, 2012).

2.1.2 Endocrine disruption, Thyroid (T)

In vitro data (T)

In an *in vitro* study using human liver fractions, Triclosan has been shown to act as selective inhibitor of glucuronidation and sulfonation of phenolic xenobiotics (Wang *et al.*, 2004), it is a powerful inhibitor of estrogen sulfonation in sheep placental tissue (James *et al.*, 2010) and in human hepatoma cells exposed to different concentrations of Triclosan, it proved to be a moderate inducer of hPXR activity (Jacobs *et al.*, 2005). Triclosan has also shown weak agonistic activity in the aryl hydrocarbon receptor (AhR)-responsive bioassay (Ahn *et al.*, 2008), indicating that it affects receptors, which are implicated in various toxic and biological responses in the body, and in addition Triclosan has been shown to alter thyroid homeostasis *in vitro* (Veldhoen *et al.*, 2006).

In vivo, human health and mammalian wildlife (T)

Effects on the thyroid hormone system have also been reported in several *in vivo* studies, as Triclosan exposure has lead to reductions in serum thyroxine (T4) levels in several rat studies. Crofton *et al.* (2007), dosed young female Long-Evans rats by daily gavage with 0, 10, 30, 100, 300, 1000 mg Triclosan/kg/day, from PND 28-31. The authors found that the three highest doses of Triclosan caused significant T4 reductions. At the highest Triclosan dose, an increase in liver weights was also seen. In young Wistar males exposed to 3, 30, 100, 200, 300 mg Triclosan/kg/day by gavage, from PND 23-58, T4 levels decreased significantly in the four highest dose groups, and thyroid histology was adversely affected at the highest dose (Zorilla *et al.*, 2009).

In a pubertal assay by Stoker *et al.* (2010) it was also shown that doses of 37.5 mg/kg and above decreased total serum T4 levels in female Wistar rats, and in a developmental study by Rodrizuez and Sanchez (2010) T4 levels in Wistar dams were significantly reduced after 10 and 50 mg Triclosan/kg/day. In female rats dosed with 0, 10, 30, 100, 300, 1000 mg Triclosan/kg/day from PND 28-31, significant T4 reductions were seen in the three highest dose levels, and it was shown that Triclosan up-regulated both mRNA expression and activity of some phase I and phase II hepatic enzymes (Paul *et al.*, 2010a).

In a developmental study by Paul *et al.* (2010b), Long Evans rats where dosed with 30, 100 or 300 mg/kg/day by oral gavage from GD 6 to PND 21, and here the highest dose caused significantly decreased T4 levels in dams on PND 22. In pups, a unique pattern of hypothyroxinemia was observed; serum T4 levels were decreased in PND4 pups whereas no significant reductions were observed on PND14 or PND21 indicating that toxicokinetic or toxicodynamic factors could have either reduced exposure or reduced the toxicological response in the offspring during the lactation period.

In a similar study design, only using more animals per group, including a lower dose of 10 mg/kg/day, adding an additional sacrifice on gestation day (GD) 20, and including more mechanistic endpoints, Paul *et al.* (2012) showed similar results. Here a significant decrease in T4 for was seen for PND22 dams at 100 mg/kg/day, and for GD20 and PND22 dams at 300 mg/kg/day. For offspring, serum total T4 levels were significantly decreased in GD20 fetuses from the 100 to 300 mg/kg/day treatment groups and in PND4 pups from the 300 mg/kg/day treatment group, but again there were no effects on serum T4 levels for PND14

or PND21 offspring. T4 reductions for dams and GD20 and PND4 offspring, and concomitant increases in PROD and UGT enzyme activity in the liver, suggested that up-regulated hepatic catabolism could contribute to the observed T4 reductions. Furthermore, and serum and liver Triclosan concentrations demonstrated greater fetal than postnatal internal exposure, consistent with the lack of T4 changes in PND14 and PND21 offspring, showing that Triclosan was only minimally transferred to the offspring through maternal milk.

This literature review shows that in all studies where rats have been exposed to Triclosan, the effects have been reductions in serum T4 levels. So even though these studies were not performed according to GLP, they are regarded as of high quality and reliable and the Triclosan that has been used in all these US EPA studies was supplied by the Registrant(s) and was guaranteed to be without toxic contaminants. Based on the weight of evidence this repeated finding of reduced T4 serum levels in Triclosan exposed animals is striking. The differences in dose-ranges at which the effect occurs, probably mostly reflect difference in sensitivity of the strains of rats that have been used, as studies where high doses of Triclosan have been needed to induce T4 reductions were all performed in Long Evans rats, whereas in Wistar rats this endpoint generally seemed to be affected at lower doses of Triclosan.

Based on the *in vitro* and *in vivo* results reviewed above, an adverse outcome pathway for the effects of Triclosan on the thyroid hormone system has been proposed by the US EPA. Here the activation of the pregnane X receptor (PXR) and/or the constitutive androstane receptor (CAR) in rat liver by Triclosan is an initiating event, leading to the effect on the circulating free T4. The activation of these receptors has been shown to result in up-regulation of hepatic phase I and phase II enzymes, leading to an increased catabolism of thyroid hormones in rats, manifested by a decrease in total T4, with a subsequent potential impact on neurological development (US EPA, 2011, Paul *et al.*, 2012).

Adverse effects in humans are correlated to low thyroid hormone levels

It is well established that severe reductions of human thyroid hormone levels during development can cause irreversible neurological effects. However, also non-clinical hypothyroxinemia (reduced maternal T4 levels) is known to be associated with adverse effects on the neurological development in children (Zoeller *et al.*, 2007, Vandenberg *et al.*, 2012). It has been shown that children of mothers, who were in the lowest 10^{th} percentile of the study population with regard to concentration of free T4, had significantly lower psychomotor development at the age of 10 months, than was seen in the reference group (Pop *et al.*, 1999), and that low maternal free T4 concentrations (hypothyroxinemia) during pregnancy was associated with altered brain development at one and two years of age, as the children performed worse in tests of motor and mental skills (Pop *et al.*, 2003). Other studies have demonstrated that children of healthy women who had very high TSH concentrations in the 17th week of pregnancy (above 98 percentile) also had significantly lower IQ levels at the age of 7-9 year (Haddow *et al.*, 1999) and that the IQ in these children was inversely correlated with maternal TSH concentration (Klein *et al.*, 2001). Several more recent studies have further corroborated the above described findings, as it has been shown that hypothyroxinemia in otherwise healthy mothers can result in adverse effects on a child's intelligence and motor development (Kooistra *et al.*, 2006, Li *et al.*, 2010), be a risk factor for both verbal and nonverbal cognitive delay in early childhood (Henrichs *et al.*, 2010), and cause behavioral problems in one to three year-old children (Ghassabian *et al.*, 2011).

Species differences in thyroid hormone homeostasis

In the existing animal database for Triclosan no developmental neurotoxicity studies are available, so while the proposed adverse outcome pathway identifies key events for Triclosan-induced hypothyroxinemia, a number of uncertainties remain as to whether the magnitude of the observed thyroid hormone alteration is sufficient to affect brain development. When extrapolating data from rats to humans for risk assessment purposes, it is important to keep in mind some important species differences. These include the toxicodynamic and toxicokinetic differences in thyroid hormone homeostasis, as well as differences in the timing of brain maturation between humans and rats.

With regard to thyroid hormone homeostasis, adult humans seem to be less sensitive to chemical-induced perturbations than adult rats, due to a high reserve capacity of thyroid hormones and the presence of high-affinity binding proteins in human serum. In rats, most T4 in serum is bound to transthyretin, which has a lower binding affinity for T4, resulting in a higher rate of T4 clearance in rats compared with humans (Savu *et al.,* 1987; Health Canada, 2012). In humans, less than 1% of T4 is freely circulating and available for destruction by liver enzymes, and it is therefore possible that humans have a greater resistance than the rat to thyroid toxicity which occurs secondary to liver enzyme activation (Health Canada, 2012), and that humans do not show reduced T4 levels as quickly as rats after exposure to thyroid disrupting chemicals. If however, exposure is prolonged or if the affinity of human T4 binding to proteins in the blood is not stronger than the signal from Triclosan to activate enhanced liver clearance, Triclosan might still cause increased T4 clearance in humans. So unless it can be proven that the rat is an unsuitable model with regard to these specific endpoints, because the differences between humans and rats in relation to the sensitivity of chemical effects on T4 clearance are qualitative rather than quantitative, the results obtained from rats with regards to T4 reduction, cannot be discarded and should be considered relevant for human health risk assessment.

Species differences in timing of neurological development

Both rodents and humans need thyroid hormones for differentiation and maturation of the central nervous system, but species differences exist in the timing of these events. While many brain maturation processes happen prenatally in humans, much more happens in the early postnatal period in the rat. Hence, the early postnatal period in rats mimics the last trimester in humans (Howdeshell, 2002). These species differences could affect the importance of the timing of correct thyroid hormone levels during brain development in the different species.

A few recent developmental neurotoxicity studies indicate that in opposition to what is seen in humans, maternal hypothyroxinemia during gestation in rats is not necessarily correlated to adverse neurological effects in the offspring (Axelstad *et al.,* 2011 a,b; Gilbert & Sui 2008). Presently, very little is however known about the consequences of thyroid hormone deficiency at specific time points during development in rats, and since brain development happens during discrete time windows, the right thyroid hormone levels at these exact time points may be very determining for the behavioral outcome. In rats some of these windows occur in the postnatal period, and it is therefore plausible that T4 levels in the offspring also need to be severely reduced postnatally for behavioral effects to occur. This hypothesis fits well with a number of rodent studies showing that postnatal thyroid hormone insufficiency was present where behavioral effects were seen after developmental hypothyroidism (Brosvic *et al.,* 2002; Noda *et al.,* 2005, Akaike *et al.,* 1991; Provost *et al.,* 1999; Axelstad *et al.,* 2008).

Epidemiology

Human data on thyroid disruption by Triclosan

A study of the thyroid hormone disrupting effects of Triclosan in humans has also been performed, and is included in the Registrant(s)'s Assessment Report on Triclosan. In this published 14-day study by Allmyr *et al.* (2009), the effects of Triclosan on thyroid hormone status were measured in 12 adult humans following exposure to Triclosan-containing toothpaste. The highest serum concentration was determined to be equivalent to a Triclosan dose of 0.1 mg/kg bw per day. No significant changes in plasma levels of either 4β-hydroxycholesterol (indicative of CYP3A4 induction) or thyroid hormone levels were seen during the exposure. These data are used by the Registrant(s) to argue that Triclosan-induced alterations in T4 levels are unlikely to occur in healthy adult humans. However, a severe limitation to this study is the very small study population and short exposure time, which limits the conclusions that can be drawn from this study. The exposure time of 14 days may be too short to impact circulating levels of thyroid hormones, as humans have reserve storage normally lasting for around a month and the small sample size of only 12 persons may not provide sufficient statistical power. However, another study including more participants and longer exposure time has recently been published (Cullinan *et al.*, 2012), and here there also seem to be no effect on thyroid function in humans exposed to Triclosan containing toothpaste.

A serious weakness by this study is the fact, that they do not have any measures of Triclosan exposure level, e.g. urinary excretion of Triclosan. Beside toothpaste, exposure sources to Triclosan include a variety of consumer products: mouthwash, deodorants, soaps, textiles (e.g. socks), toys and liquid dishwashing soap (Calafat *et al.*, 2008). In an American biomonitoring study it was found that 75% of the study population had measurable levels of Triclosan in the urine (Calafat *et al.*, 2008). As the participants in the study by Cullinan *et al.* may have been exposed to Triclosan from other sources than toothpaste, and there has not been distinguished between high vs. low Triclosan exposure, this study is not suitable for drawing conclusions on an overall effect of Triclosan on thyroid function. Another limitation of the study was that the selected subjects were predominantly older males and so may not reflect the population of concern for thyroid hormone changes, as subclinical thyroid hormone changes in women of child-bearing age may result in irreversible neurological effects on the developing child.

Summary of Registrant(s)'s comments and responses to comments.

In their comments on 'In vivo, human health and mammalian wildlife (T) Effects on the Thyroid axis', the Registrant(s) put forward the argument that since Triclosan reduces thyroid hormone levels by activating hepatic catabolism, it is unclear if this mechanism has any relevance to the human situation. Furthermore, since it is the Registrant(s)'s experience that in developmental neurotoxicity studies morphological changes are more sensitive than behavioural tests, and such changes have not been seen after Triclosan exposure, the Registrant(s) find it unlikely that the proposed study design for a DNT study is appropriate.

Various arguments were brought forward and are presented here in an edited form, together with the corresponding reply of the eMSCA.

a) The Registant(s) commented on the human relevance of thyroid disrupting mechanisms. Paul *et al.* (2010) found decreased T4 levels in rats exposed to Triclosan together with evidence for upregulation of hepatic catabolism, with the initiating event most likely being the activation of hepatic CAR and PXR receptors. Hepatic catabolism, in turn, is thought to increase T4, which leads to the observed T4 depletion. Because enzyme induction studies in rodents often fail to predict enzyme induction in humans (Cassarett *et al.*, 2007), it is presently unclear if this

mechanism has any relevance to the human situation.

The eMSCA noted that because of species differences in e.g. enzyme induction, extrapolatation of results obtained with rodents to humans, should be done with caution. However this is not a reason to discard the rodent Triclosan findings or to conclude that they are not at all relevant for humans. Triclosan has been shown to act as a moderate inducer of human PXR activity (Jacobs *et al.*, 2005), indicating that this could also be an initiating event in an adverse outcome pathway in humans. Furthermore, other classes of compounds (polychlorinated biphenyls (PCB), brominated flame retardants) exist, which in rats reduce thyroid hormone levels by increasing liver catabolism via the PXR/CAR pathway, and where associations with altered thyroid hormone function in humans have been observed. Together these results indicate that such a mechanism of thyroid disruption could indeed be relevant for human risk assessment.
Finally the Registrant(s) also provided a range of specific comments to other parts of the draft decision relating to *in vitro* data on endocrine related tests/ endpoints. These comments and the reponses to them by the eMSCA constitute the background for the decision and the related information requests (c.f. Annex 2).

b) The Registrant(s) commented on the relevance of the proposed investigation of reproductive endpoints] In the Zorilla *et al.* (2009) study T4 was as low as 20% of controls, with no effect on androgen-dependent tissues. Another Triclosan study found no effect on sperm morphology, motility, cauda sperm counts, testes sperm counts or the estrous cycle stage (Jia-Long *et al.*, 2013). In this study, T4 was knocked down to less than 50% of the control values. Numerous studies with repeated exposure in six species with doses up to 900 mg/kg bw/day failed to show effects on the gonads or the thyroid gland, and the two generation study in rats revealed no adverse effects on reproductive development.

   The eMSCA observed that in studies by Zorilla *et al.* and Long *et al.*, and the mentioned repeated exposure studies in different species, Triclosan dosing was not performed during the critical pre- and postnatal periods of reproductive development, which could account for the lack of effects on the reproductive system. Since T4 reductions in adult rodents, to our knowledge are not known to produce adverse effects on reproductive function, these results do not make investigation of reproductive effects in perinatally exposed offspring any less relevant. Furthermore in studies from the US EPA investigating the effects of Triclosan on female reproduction in a pubertal study, Triclosan advanced the age of onset of vaginal opening and increased uterine weight at 150 mg/kg, which is indicative of an estrogenic effect of Triclosan (Stoker *et al.*, 2010) and it was shown in two studies that much lower doses of Triclosan (≥4.7 mg/kg) potentiated the effects of estradiol in uterotrophic assays (Stoker *et al.*, 2010, Louis *et al.*, 2013). With regards to gonadal development in the 2-generation study, reproductive organ weight, histopathology and semen quality were not investigated.

c) The Registrant(s) commented on the relevance of the proposed developmental neurotoxicity study. There are several studies in the public domain that share similarities with the study design for the developmental neurotoxicity study requested in the decision. Three studies, published by Axelstad et al. (2008, 2011a,b) investigated developmental neurotoxicty with Mancozeb, 2-ethylhexyl trans-4-methoxycinnamate (OMC) and propylthiouracil (PTU). T4 levels were depressed in all three studies, but Axelstad *et al.* only found effects on the thyroid gland and on behaviour in the PTU study. Additional studies listed in the decision found developmental neurotoxicity of thyroid disrupting chemicals that was

accompanied by decreased litter size, increased pup mortality, slower body weight development, increase in thyroid weight with concomitant histological abnormality and increase in relative brain weight. Especially the retarded development of the pups appears to be a consistent pattern in T4 depressed rats. In addition, histopathological changes and body/ organ weight effects appear to precede effects in behavioural tests, and it is the Registrant(s)'s experience that morphological changes are more sensitive than behavioural tests. Hence, in the absence of hints in studies with Triclosan on adverse effects triggered by T4 depression, it seems unlikely that the proposed study design for a developmental neurotoxicity study with Triclosan is appropriate.

The eMSCA observed that a common effect seen in offspring that have been exposed to compounds which reduce their thyroid hormone levels in the postnatal period, is decreased body weight gain and increased thyroid size/altered histology - effects that have been seen in several rat studies investigating the effects of potent thyrotoxic compounds like PTU or PCB). If, on the other hand, thyroid hormone levels in the offspring are not severely decreased during the first few postnatal weeks (for instance due to limited milk transfer), decreases in body size and neurobehavioural effects would not be expected. And this is exactly what has been observed in a number of studies with compounds that have reduced T4 levels in adult and pregnant rat dams, but not in young offspring exposed indirectly through the dam, as for instance seen with Mancozeb (Axelstad *et al.*, 2011a), OMC (Axelstad *et al.*, 2011b), and perchlorate (Gilbert & Sui 2008). Since Triclosan exposure reduces T4 levels in adult and prepubertal rats, but not in offspring during the first postnatal weeks (Paul *et al.*, 2010, 2012), a developmental study with indirect Triclosan exposure (i.e. only via lactation), would in our opinion not be expected to result in adverse effects on the thyroid glands or in behavioural effects in rat offspring. If however postnatal T4 reductions were present (which would be achieved by direct dosing), effects on the developing nervous system are in our opinion likely to occur. Employment of direct Triclosan dosing in a developmental rat study is requested in the decision, in order to compensate for the differences between exposure routes of Triclosan in rats and humans at similar life stages. With respect to brain development, rat offspring are born less mature than human babies, i.e. the last trimester in a human pregnancy corresponds approximately to the first 10 day after birth in a rat pup. Since Triclosan transfer to maternal milk is limited, direct dosing of the pups in the postnatal period is essential in order to cover the last part of human foetal brain development. Since maternal T4 levels during pregnancy seem to be determining for the neurological development of the child in humans (as reviewed in Hartoft-Nielsen *et al.*, 2011), compounds that have the ability to reduce thyroid hormone levels, could potentially affect human neurodevelopment.

Since Triclosan dosing of the dams does not reduce T4 levels in the pups during the first postnatal weeks, a developmental neurotoxicity study with indirect dosing of pups (i.e. through maternal milk only) would indeed be inappropriate, as the eMSCA would not expect any adverse neurodevelopmental effects. This is why the decision states that the DNT study shall be performed with direct dosing of the pups in the postnatal period, to cover all sensitive periods of brain development.

d) In their comments on 'relevance of the rat', the Registrant(s) were of the opinion that the fact that oral absorption, metabolism and excretion are comparable in hamsters and humans suggests that the hamster is the most relevant species for human risk assessment, and that the requested studies are not justified to provide a conclusive answer to address any potential concerns about possible endocrine disruption caused by Triclosan.

Pharmacokinetic data suggest that enterohepatic circulation occurs in the rat and mouse but appears to be absent in humans and hamsters. All species tested so far exhibit complete metabolisation of the parent compound to the glucuronide and sulfate conjugate. In a number of species, including the rat, the sulfate conjugate dominates the glucuronide with the feces being the primary route of excretion. In hamsters and humans the glucuronide conjugate predominates in urine which is the primary route of excretion of these two species. The elimination half-lives in hamsters and humans are additionally similar (but different from those in the rat and mouse). The fact that oral absorption, metabolism and excretion are comparable in hamsters and humans suggests that the hamster is the most relevant species for human risk assessment. As stated above, the induction of phase II catabolism in rats may have no relevance to humans, which makes it necessary to clarify whether this initiating event of the adverse outcome pathway has any relevance to the human situation. Based on the available data set for this substance, the requested studies are not seen as justified to provide a conclusive answer to address any potential concerns about possible endocrine disruption caused by Triclosan.

The eMSCA noted that the argument that the hamster is the most appropriate species for extrapolation to human with regard to Triclosan, has previously been presented in a supplementary submission to the opinion on Triclosan by the Scientific Committee on Consumer Products (SCCP). Triclosan from January 2009. Here the applicant asked the SCCP to further consider differences in kinetics between species, since Triclosan, in the form of conjugates, undergoes extensive enterohepatic recirculation in rats and in mice, but not in hamsters and humans. However, the SCCP saw no reason to favor hamster data and disregard rat data in its evaluation of Triclosan, and the SCCP in their 2011 addendum did not want to discard the data obtained in rats. The eMSCA concurred with the SCCP, and found that the rat data are also relevant for this evaluation Triclosan. The Registrant(s) have not documented that the rat is an irrelevant model to use for human risk assessment, but only states that the induction of phase II catabolism in rats may have no human relevance. In order to reject rat data, proof that these are of no relevance for humans is needed. Since counter-arguments have been presented to all the comments raised by the Registrant(s), and no significant new information has been put forward to justify not requesting the study, only minor changes have been implemented in the decision and the requested study is maintained therein.

Summary of proposals for amendment made by MSCAs, Registrant(s)'s comments on them and responses to Registrant(s)'s comments.

Two MSCAs made PfAs:

1) One MSCA supported the proposed study, but had concerns regarding the animal welfare implications some specific investigations being requested and addressed three specific issues in this regard:

   Sub-Lingual blood sampling of the dams:

   "In our opinion, Directive 2010/63 would normally require that anaesthesia is used for this procedure unless this is more traumatic for the animal or prevents the scientific goals of the study being achieved. What is the justification for the use of unanaesthesitised animals? Without good justification, anaesthesia should be used".

   Blood sampling pups proposed on PND 10-14

"Blood sampling of animals this young is unusual, as most vessels will be small in terms of access and also blood volume available is also small. Therefore, the route of sampling would be important – saphenous vein (lateral leg vein) might be acceptable as might manage the tail. Currently, the recommended sampling volume for a rat is 10% for a single sample (unless terminal). For a 10 day old pup, at around 30g the circulating blood volume is 60ml per kg and we estimate this to be 0.18ml, per pup which is probably more than will be obtained in practice. If the scientific aims can't be achieved with this volume, this investigation should not be required.
We suggest adding an additional sentence in section II of the draft decision, last paragraph of request No 2: Blood sampling is limited to a maximum of 10% of the estimated blood volume".

Dosing of 3-4 day old pups

"The recommended dose volume is 0.01ml per 10g; we are concerned that dosing of these young animals at the required volume may not be technically achievable. Furthermore, we are concerned that the severe stress associated with dosing of pups pre weaning may well compromise the scientific aims of the study, and ask that the need for this procedure is reconsidered".

2) Another MSCA made the following comment:

"Moreover, the OECD Guideline 451 (Carcinogenicity Studies) dated 1981 indicated abnormal spermatogenic cells, absent or reduced numbers of spermatozoa, and partial depletion of one or more generations of germ cells in males treated with 250 mg/kg/bw of Triclosan. Finally recent published literature[2] indicates that Triclosan exhibits a tendency to accumulate in the epididymis and shows sperm toxicity in male sprague-dawley rats.
Therefore, male fertility is a point that needs to be evaluated carefully".

The following responses were provided by the eMSCA:

1) Animal welfare issues in relation to the requested Enhanced Developmental Neurotoxicity Study design:

   Sub-Lingual blood sampling of the dams:

   The important message in the study design outlined in the decision is that blood samples should be taken from the dams during gestation, for measurements of thyroid hormone levels. If blood sampling is performed during anastesia there is a risk of animal loss. Some research laboratories therefore use a procedure where blood is taken from the tongue of the adult rats without anaesthesia. This procedure is quick and relatively easy to learn and does not seem to stress the animals. Should the performing laboratory however prefer to use another procedure for obtaining blood samples from pregnant dams for thyroid hormone measurement, this does not constitute a problem. Hence the wording in the decision text has been slightly revised to make it clear that an adequate procedure minimizing any discomfort for the animals should be chosen when taking blood samples. The procedure mentioned above is now mentioned as an option.

[2] Triclosan exhibits a tendency to accumulate in the epididymis and shows sperm toxicity in male sprague-dawley rats. Lan Z1, *et* al. Environ Toxicol. 2013

Blood sampling pups proposed on PND 10-14:

The text in the draft decision was not specified clearly enough. It has been revised to: "During the postnatal period (preferably between PND 10 and 14) when the pups are being directly exposed to Triclosan, 1-2 pups per litter should be sacrificed and trunk blood should be used for thyroid hormone measurements".
The eMSCA was of the opinion that this clarifies the issue, and makes the additional sentence recommending a maximum draw of 10% of the blood volume irrelevant.

Dosing of 3-4 days old pups:

Due to the reasons already outlined in the draft decision, the eMSCA found that direct dosing of the pups in this study is crucial. Furthermore, amongst other publications, a recently published study in the US-FDA clarity-BPA project (Delclos et al 2014) has used a similar dosing procedure, for investigation of the effects of perinatal BPA exposure. In the Delclos et al (2014) paper the following procedure is outlined:

"Direct oral gavage dosing of the pups started on PND 1, after the litter was culled. Pups were weighed and dosed daily until the scheduled day of removal (PND 15 or PND 21) or until the day prior to their scheduled removal (PND 90 ± 5). For pups younger than PND 5, the gavage needle did not enter the esophagus. Naïve control dams and F1 pups were weighed daily and returned to their cages after any scheduled observations were conducted. Pups were housed individually after weaning, consistent with the FDA guidance in place at the time the experiment was initiated (USFDA, 2007). "

This example from another targeted concern driven testing indicates that the proposed procedure on Triclosan was technically achievable in the large Bisphenol A study. The study included both a naïve (not dosed) and a vehicle control. There were no differences in body weights between these two groups indicating that the direct dosing of the pups did not affect body weight. Furthermore, the researchers involved in the CLAITY-BPA project must have assessed that choosing this relatively time-costly route of exposure was the right way to achieve their scientific aims, in spite of the potential stress experienced which may result from the direct dosing procedure of the pups. The eMSCA was of the opinion that this is also the case when studying the behavioural and reproductive effects of Triclosan. In relation to the dosing volume of pups the decision was revised so that it now refers to a dosing procedure and a dosing solution volume in e.g. corn oil, in accordance with paragraph 30 of OECD TG 443 and with a dosing volume as low as possible, in accordance with good animal welfare practice.

2) Careful evaluation of male fertility based on the results in an OECD TG 451 study:

The text of the decision has been slightly revised accordingly (c.f. below, section 2.1.1.b)

Summary of Registrant(s)'s comments to the proposals for amendment[3] made by MSCAs.

[3] In relation to the reference numbers: In this decision the reference numbers of the document containing the Registrant(s)' comments to PfAs have been maintained. The reference list is available in Annex 3.

1. The Registrant(s) agreed with the PfA in terms of animal welfare concerns with the proposed study design. He furthermore repeated that: *"a study by Axelstad et al. that was funded by the Danish Environmental Protection Agency, the evaluating member state of this dossier evaluation, and submitted to Food and Chemical Toxicology on April 18th, 2013, i.e. 2 days prior to issuing the draft decision on substance evaluation, employed a very similar study design as that proposed in the amended draft decision (DD) [1]. In the first part of this study, time-mated Wistar rats were dosed as described for the requested study in the DD, however, anesthesia was used to draw blood from the tail vein. In the second part of the study, pups were dosed directly from PND 3 to PND 16 and trunk blood was used for total T4 analysis following sacrifice. Consistent with other studies conducted by the US EPA, a dose-dependent decrease of T4 was seen in dams. In offspring, T4 was only reduced in directly dosed animals. Because it was obviously feasible to use anesthesia during blood sampling, a justification for the deviating procedure proposed in the DD is missing".*

2. *"In addition, the first part of the study by Axelstad et al. revealed no effects on thyroid weight in dams and offspring, anogenital distance, nipple retention or prostate weight and morphology. These results are in line with repeated dose studies in the Registrant(s)'s data base. Several studies in the rat conducted with United States Pharmacopeia (USP)-grade material found no effect on the morphology of the prostate, testes or epididymis with doses up to ca. 300 mg/kg bw (90-day treatment) or up to ca. 150 mg/kg bw (two year treatment). In addition, no weight changes were observed for testes or epididymis in a two generation study [2] and the number of corpora lutea was unaffected by Triclosan treatment [3]. This information, although not explicitly summarized, was submitted as part of the technical registration dossier and can be found by carefully reading the robust study summaries of the relevant studies. An evaluation by Mangelsdorf et al. (BAuA) found a strong correlation between histopathology data, organ weights and male fertility. The authors concluded that these data from repeated dose studies may be used to identify adverse effects on male reproduction [4]. Therefore, large parts of the requested study that concern the extended one generation part as laid out in the DD are a mere repetition of already existing studies."*

3. *"Human data exist that show a lack of effects on thyroid hormone homeostasis. This was demonstrated in several studies involving the use of Triclosan containing toothpaste over 4 years in a placebo-controlled study [5] or following short term exposure [6] and in a cross-sectional study with the help of the 2007-2008 urinary data from the National Health and Nutrition Examination Survey (NHANES) in the USA [7]. No effects on important parameters such as free and total T4, T3, THS or thyroglobulin were found following direct treatment with Triclosan containing products."*

4. *"The retrospective study using NHANES data revealed a positive association of urinary Triclosan and T3, while T3 concentrations were still within published ranges. Nuclear receptor binding studies revealed that Triclosan interacts with human and rat receptors through divergent pathways to induce hepatic catabolism with downstream effects on thyroid hormone homeostasis [8]. This together with the considerably higher buffering capacity of the human thyroid hormone system may explain why the effects seen in rats are not evident in humans.".*

5. *"Moreover, although it is known that disruptions in thyroid function during pregnancy can cause neurological deficits in offspring, a threshold seems to exist and severe*

reductions in thyroid hormones are necessary to exert an adverse effect [9, 10]."

6. *"In summary, conducting an animal study to further investigate the potential hazard of developmental neurotoxicity would not further the science and would be an irresponsible and excessive use of experimental animals."*

7. *"MS commented on a study by Lan* et al. *[10] that identified sperm toxicity and accumulation of Triclosan in reproductive tissue in the context of the hamster carcinogenicity study performed in 1999 [12]. Histopathological changes in the testes and sperm effects go hand in hand [4], in fact histopathology of the testes was shown to be the most sensitive parameter, followed by weight of the reproductive organs and effects can be seen in repeated dose studies as early as after four weeks continuous treatment. Repeated dose studies with Triclosan as documented in the technical registration dossier are available in several species with up to lifetime treatment [13-19] or over multiple generations [2] which revealed no histopathological or weight changes in any of the above mentioned reproductive organs. It can therefore be concluded that United States Pharmacopeia (USP)-quality Triclosan does not cause these effects.The incidence of regressed testes in the hamster lifetime bioassay is thought to be secondary to a treatment related decrement in body weight gain [12]. This is species specific to the hamster as a seasonal breeder that has evolved certain mechanisms for the spontaneous regression of testicular tissue at times of the year when breeding is not possible [20]. The effects seen at 250 mg/kg can therefore not be attributed to Triclosan treatment, a fact that was accepted* [redacted] *during the assessment by the SCCS (Scientific Committee on Consumer Safety)* [redacted]

Responses by the eMSCA:

1. To take account for the PfA concering animal welfare in the requested Enhanced Developmental Neurotoxicity Study, the text in the decision was revised in the following way:" All blood sampling should be performed to minimize animal distress. A possibility is blood sampling from the tongue of the pregnant rats without anaesthesia, a method which has the advantage that potential animal loss because of use of anaesthesia is avoided. Should the performing laboratory however prefer to use another procedure for obtaining blood samples from pregnant dams, this should be done in a way to minimize animal distress as mentioned above".

It is noted that none of the new comments no. 2 to 6 from the Registrant(s) concerns the PfA from the MSCA in question. In principle they were therefore outside the scope of commenting at this stage of the decision making procedure. Nevertheless the following responses can be provided (each of the responses below refers to the same comment running number):

2. The Registrant(s) have already previously commented on the lack of reproductive effects in some of the performed Triclosan studies. As also previously replied by the eMSCA, in these studies Triclosan dosing was not performed during the critical pre- and postnatal periods of reproductive development, and therefore they do not provide sufficient information to safely assess lack of reproductive effects. With regard to gonadal development in the 2-generation study, reproductive organ weight, histopathology and semen quality were not investigated. In contrast to the conclusions reached by Mangelsdorf *et al.*, Janer *et al.* (2007) in their retrospective analysis of added value of the two-generation study found that if no reproductive effects were seen in a subchronic study, this did not exonerate the substance for

reproductive effects. Generally the two-generation study had little impact on changing the overall NOAEL available from a subchronic (or chronic) studies, but had a marked impact on classification for toxicity to fertility, as about one-third of the substances shown to be toxic to fertility in the two-generation study did not show any sign of that in the 90-day study. Furthermore in studies from the US EPA investigating the effects of USP grade Triclosan on female reproduction in a pubertal study, Triclosan advanced the age of onset of vaginal opening and increased uterine weight at 150 mg/kg, which is indicative of an estrogenic effect of Triclosan (Stoker *et al.*, 2010). It was also shown in two studies that much lower doses of Triclosan (≥4.7 mg/kg) potentiated the effects of estradiol in uterotrophic assays (Stoker *et al.*, 2010, Louis *et al.*, 2013).

3. The limitations of the studies with direct Triclosan exposure have also previously been discussed in the eMSCA's responses to the original comments from the Registrant(s) on the draft decision.

4. The fact that NHANES data showed a correlation between Triclosan and T3 concentrations in adolescents, and that studies show nuclear receptor binding of Triclosan in both rodent and human cells, in our opinion only exasperates the need for further investigation of the endocrine disrupting effects of Triclosan.

5. No, in the quoted studies subclinical hypothyroxinemia in pregnant mothers was associated with adverse neurodevelopmental outcomes in the children. Hendrichs *et al.* (2010) showed that both mild and severe maternal hypothyroxinemia was associated with a higher risk of expressive language delay, across all ages of children. And Pop *et al.* (1999) who investigated healthy women from iodine sufficient areas found that in the women with the lowest 10th percentile fT4 concentrations, a positive correlation could be seen between the mothers' fT4 concentration at 12 weeks' gestation and their children's Bayley Psychomotor Developmental Index scores. These, and similar results from other publication indicate that thyroid hormone levels do not need to be severly reduced during pregnancy in order to see adverse effects on fetal brain development.

6. For the reasons stated above, the eMSCA did not agree with this conclusion of the Registrant(s), and finds that the study outlined in the draft decision would contribute to a better understanding of the potential endocrine disrupting effects of Triclosan - both in regard to reproductive development and adverse effects on the developing brain.

As none of the comments 2-6 from the Registrant(s) were concerning the PfA from MSCAs, these comments were not taken into account in the decision making.

7. Effects on male fertility are not the main concern with regard to Triclosan exposure (as also outlined above). However, since there are some indications of effects, the eMSCA agreed with the second above mentioned PfA from a MSCA that semen quality and male reproductive organ weights and histopathology should of course be carefully evaluated in the proposed study – especially since they were not investigated in the performed two-generation study.

The decision was not revised based on the Registrant(s) comments to the PfAs (c.f. above points 1 and 7)

Conclusions concerning endocrine disruption and human health and mammalian wildlife

The text of the decision has been revised to reflect that the proposed study design has also been used before for other targeted testing on substances of concern and that the blood sampling should be performed to minimize any potential distress (i.e. blood sampling from the tongue of the adult rats without anaesthesia is just one possibility which however has the advantage that potential animal loss because of use of anaesthesia is avoided).

In respect to the second PfA above the eMSCA agreed and the decision was revised accordingly (see text revision in section 2.1.1.b)

Since the available data for Triclosan raises concern on endocrine disruption with regard to both estrogenic and thyroid mode of action, a testing strategy is proposed which could determine adverse outcomes of both these types of endocrine disruption. Exposure to estrogenic compounds could give rise to adverse effects like altered anogenital distance, altered weight and histopathology of reproductive organs, altered timing of sexual maturation, altered estrous cyclicity and decreased semen quality, whereas thyroid disruption during development could result in adverse neurobehavioral effects.

In their comments on the summary of the draft decision the Registrant(s) noted that since the information coming from open literature was in conflict with in vivo data resulting from GLP guideline studies, ECHA could not judge whether Triclosan acts as an endocrine disrupter or not. Further the Registrant(s) stated that they had now provided additional in vivo data on both fish and amphibians, which in their view allows a clear judgment on the endocrine activity of Triclosan.

The eMSCA concluded that that an additional Triclosan study is requested in order to clarify if Triclosan acts as an endocrine disrupter on the gonadal axis, and to investigate whether the thyroid hormone reductions observed in numerous rat studies lead to adverse neurobehavioural effects. Furthermore the newly submitted data on fish and amphibians are of limited relevance for the assessment of Triclosan as an endocrine disrupter with regards to human health.

Therefore, the following test is required which consists of a merging of the two OECD guidelines which are most appropriate for answering these concerns, namely the extended one generation reproductive toxicity study (TG 443) and the developmental neurotoxicity study (TG 426):

> Enhanced Developmental Neurotoxicity Study, OECD Guideline for Testing of Chemicals, No. 426, OECD, Paris) with relevant elements of Extended One-Generation Reproductive Toxicity Study, OECD Guideline for Testing of Chemicals, No. 443, OECD, Paris.

The requested study shall include the oral OECD TG 426 design, but should additionally include several endpoints which are mandatory in the extended one generation study, in order to take into account the concerns for possible estrogenic action of Triclosan. Therefore, the TG 426 study should also include measurements in the offspring of: anogenital distance, weight and histopathology of reproductive organs, including a quantitative evaluation of primordial and small growing follicles, as well as corpora lutea, estrous cyclicity and semen quality, as detailed in the OECD TG 443. The OECD TG 416 study performed by the Registrant(s) in 1988 did not include any of these measurements because the former version of this TG did not include these measures.

Furthermore the design of the currently requested developmental neurotoxicity study should include some additional endpoints to address some effects which are especially relevant for Triclosan with regard to thyroid disruption. Results showing that maternal dosing with

Triclosan in the pre- and postnatal period leads to reduced T4 levels in the offspring on GD20 and PND 4, but not on PND 14 (Paul *et al.*, 2010, 2012) indicate that Triclosan is not transferred into maternal milk to any significant degree. Furthermore exposure to a thyroid disrupting chemical during the entire period of brain development (i.e. for rats as opposed to humans both pre- and postnatally) is important in the rat study for simulating human exposure during the entire period of pregnancy.

Therefore direct dosing of the pups is requested as also stated in OECD TG 426 for substances where pharmacokinetic information exists to support this. Hence the females shall be exposed by oral gavage during pregnancy and early gestation until the direct dosing of the pups starts and the offspring shall be dosed directly during the entire postnatal period, i.e. from the 3rd or 4th day after birth until weaning. The reason why offspring exposure does not have to begin immediately after birth, is that results from Paul *et al.* (2012), show that on PND 4 offspring still have Triclosan amounts in the blood and liver which are similar to levels seen at GD 20, and that T4 reductions on PND 4 are also similar to those seen on the day before birth. In the beginning of the dosing period it may for practical reasons be necessary to use a micropipette for administering Triclosan in a solution into the mouth of the offspring. Exposure shall continue using oral gavage when the size of the pups allows for this procedure to be used. The dosing procedure shall follow the procedure as described in paragraph 30 of OECD TG 443 and in accordance with good animal welfare practice, especially since direct dosing of pups is not a frequently used route of administration.

The study shall furthermore be performed in Wistar rats because this strain of rats based on existing information seems more sensitive to the effects on Triclosan compared to other rat strains.

The dose levels for the dams shall be 30, 100 and 300 mg/kg bw/day, because these levels have been tested previously and have shown effects on maternal T4 levels. For the direct dosing of the offspring the dose level shall be 15, 50 and 150 mg/kg bw/day. If the Registrant(s) need to perform a range-finding study and results from such a study should indicate the need for modifying the proposed dose levels, this is acceptable provided that robust scientific justification is given.

The study shall also include additional measurements of T4 in all the dams during gestation, preferably around GD 15. All blood sampling shall be performed to minimize animal distress. A possibility is blood sampling from the tongue of the pregnant rats without anaesthesia, a method which has the advantage that potential animal loss because of use of anaesthesia is avoided. Should the performing laboratory however prefer to use another procedure for obtaining blood samples from pregnant dams for thyroid hormone measurement, this shall be done according to a relevant procedure, which minimizes any animal stress. 1-2 pups per litter shall be sacrificed for blood sampling and trunk blood shall be used for thyroid hormone measurements. The measurements of T4 levels in the offspring shall be taken at a point in time when they are being directly dosed, preferable between PND 10 and 14. This sample shall be retrieved to make sure that the offspring have been exposed enough to Triclosan in the postnatal period to lower their T4 levels, which may cause behavioural effects later in life.

3. Fish Sexual Development Test (FSDT, test method: OECD TG 234,) with zebrafish or Japanese medaka.

3.1. Concerns for endocrine disruption in fish

As described for the human health part above it is clear that Triclosan impacts the thyroid

hormone system in rats. Such effects are relevant to wildlife mammalian species also and the proposed study above may provide a direct evidence of whether Triclosan as indicated does cause adverse reproductive (including neuro-developmental) effects in rats. If so, this may also be relevant for mammalian wildlife species.

The available *in vitro* data (c.f. section on human health and mammalian species) indicates that Triclosan may posess androgenic, estrogenic and thyroid hormone activity and it is of course relevant to take account of related available information on aquatic species.

Robust study summaries of the REACH registration dossier as well as of relevant published open literature were reviewed in relation to available information about endocrine disruption properties of Triclosan for aquatic animals.

3.1.1. Fish

No effects of Triclosan were seen on newly hatched and adult fathead minnow *Pimephales promelas* during 12- and 21 days of exposure respectively (Schultz *et al.*, 2012). At the end of the exposure, larvae were assessed for growth and predator-avoidance performance, and a subset of mature fish was assessed for plasma vitellogenin induction, expression of secondary sexual characteristics, relative size of liver and gonads, and histopathological changes to both organs. The remaining exposed mature fish were placed in breeding pairs of one male and one female fathead minnow from the same treatment to assess their ability to defend a nest site and reproduce. Exposure to Triclosan caused no significant changes to larval or mature fish in relation to the investigated endpoints. It should though be noted that the highest test concentration was 449 ng/l.

The effects of Triclosan on the early life stages and reproduction of medaka (*Oryzias latipes*) were investigated (Ishibashi *et al.*, 2004). The 96-h median lethal concentration value for 24-h-old larvae was 602 µg/l. The hatchability and time to hatching in fertilized eggs exposed to 313 µg/l for 14 days were significantly decreased and delayed, respectively. An assessment of the effects of a 21-day exposure period on the reproduction of paired medaka showed no significant differences in the number of eggs produced and fertility among the control and 20, 100 and 200 µg/l treatment groups. Hepatic vitellogenin was increased significantly in males treated with 20 and 100 µg/l. In the F1 generations, although the hatching of embryos in the 20 µg/l treatment showed adverse effects, there was no dose–response relationship between hatchability and treatment levels. These results suggest that Triclosan has high toxicity on the early life stages of medaka, and that the metabolite of Triclosan may be a weak estrogenic compound with the potential to induce vitellogenin in male medaka but with no adverse effect on reproductive success and offspring.

Japanese medaka fry (*Oryzias latipes*) (Foran *et al.*, 2000) were exposed for 14 days beginning 2 days post-hatch to Triclosan (100, 10, 1 µg/l), 17β-estradiol (E2; 1 µg/l), or a solvent control (ethanol). Two months post-exposure, the phenotypic sex of each adult was assessed visually using sexually dimorphic fin shape and size. Triclosan treatment did not skew the sex ratio of animals grown to maturity. The 100 µg/l group containing 64% males was not significantly different from the 47% male ethanol-treated group. However, 1 µg/l E2 produced 92% females, which was significantly different than controls. As expected, males had significantly longer dorsal and anal fins than females in each treatment group. Among females, there were no differences in fin lengths between the treatment groups. Among males, animals treated with 100 µg/l Triclosan had longer dorsal and anal fins than those treated with 10 µg/l the measurements of both Triclosan-treated groups overlapped the measurements from control males. A slight increase in the length of the dorsal fin and anal fin could indicate weak anti-estrogenic or androgenic effect but the evidence in this

study was not sufficient to determine if Triclosan acts as an endocrine disruptor to disrupt development of fish.

Raut *et al.* (2010) tested the hypothesis that Triclosan acts as an endocrine disruptor (ED) in fish. Mature male western mosquitofish, *Gambusia affinis*, were exposed to Triclosan concentrations of 100, 200, and 350 nM (29.0, 57.9, and 101.3 µg/l) for 35 d by a static renewal method. Induction of vitellogenin gene expression and reduction in sperm count were quantified as biomarkers of endocrine disruption. Mean sperm counts were significantly reduced in the 350 nM Triclosan group compared with the solvent control. Vitellogenin mRNA expression was significantly increased (26.7-fold compared with the solvent control) in male mosquitofish exposed to Triclosan at 350 nM.

None of the mean gonadosomatic index (GSI) values for the Triclosan treatment groups differed significantly from that of the solvent control. The mean hepatosomatic index (HSI) value of the male mosquitofish exposed to 350 nM trioclosan was significantly greater than that of the solvent control. In conclusion, this study shows that male mosquitofish exposed to Triclosan for 35 d at concentrations approximately 100 times greater than those usually found in the environment showed evidence of endocrine disruption

Conclusions on fish

In the ecotoxicologal studies on fish, Triclosan caused vitellogenin induction in one study and a combination of vitellogenin mRNA induction and decreased sperm-counts in another study. Vitellogenin- and vitellogenin mRNA induction in fish are associated with an estrogenic mode of action, where decreased sperm counts could also be caused by anti-androgens or systemic toxicity. Triclosan is regarded as a suspected ED in fish because vitellogenin- and vitellogenin mRNA induction informs about estrogenicity but the links to the observed adverse effects are not fully conclusive. The biological significance of the decreased sperm counts observed in one study with fish is not fully clear because the fertilization rate was not investigated and therefore the link to an adverse effect on reproduction was missing. Test data relating to any specific mode of action, including endocrine activity, was also absent in this study.

Since the available data for Triclosan raises concern on endocrine disruption in fish with regard to estrogenic mode of action, OECD TG 234 can be used to obtain data about mode of action and its link to adverse effect, e.g. vitellogenin levels (and secondary sex characteristics in medaka) and phenotypic sex ratio. The optional endpoint gonadal histopathology should be included as mandatory because decreased sperm counts have been observed in a previous study and because this type of effect can be investigated by inclusion of histopathology investigations in the study. OECD TG 234 is also able to address other ED concerns i.e. androgenic mode of action (MoA) (reported *in vitro* by Chen *et al.* (2007)) as well as steroidogenesis inhibiting MoA. In section 7.1.4 conclusions are drawn on required fish testing.

3.1.2 Amphibians

Available information suggests both thyroidal and anti-androgenic effects of Triclosan in amphibians but a firm link between these T and A hormonal activities and adverse effect have not yet been established. Further information may be requested at a later stage as a follow up to the tests requested in this decision.

3.2. Concern in relation to type of endocrine activity

3.2.1. Estrogenicity (E)

Vitellogenin induction has been observed in male Japanese medaka (*Oryzias latipes*) after 21 days exposure to 20 µg Triclosan/l (Ishibashi *et al.*, 2004). Raut *et al.* (2010) observed decreased sperm-counts at 101 µg Triclosan/l in the western mosquitofish, *Gambusia affinis* (NOEC 57.9 µg Triclosan/l).

These effects are suggestive of an estrogenic action of Triclosan in fish.

3.2.2. Androgenicity (anti) (A)

Decreased sperm counts at 101.3 µg/l observed in fish (Raut *et al.*, 2010) could be caused by an anti-androgenic action of Triclosan but it could also be caused by systemic toxicity. Matsumura *et al.* (2005) observed lowered vitellogenin concentrations and testosterone levels in *Xenopus laevis* after intraperitoneal injection of 4, 40, and 400 µg/g body weight of Triclosan.

These effects are suggestive of an anti-androgenic action of Triclosan in fish and amphibians.

3.2.3. Thyroid hormone activity and disruption

As mentioned above thyroid gland hypertrophy (LOEC 0.3 µg/l) and decreased follicle cell height (LOEC 1.3 µg/l, NOEC 0.3 µg/l) was observed in the frog *Xenopus laevis* (Fort *et al.*, 2011b; Helbing *et al.*, 2011a,b,c). Furthermore Marlatt *et al.* (2013) observed disrupted coordination of postembryonic tadpole development in the Pacific tree frog (*Pseudacris regilla*) in a 21 d adapted AMA study (OECD TG 231).

These effects indicate interference of Triclosan with the thyroid hormone system in amphibians but currently it cannot be concluded that the thyroidal effects also causes adverse effects, i.e. that Triclosan causes endocrine disruption in amphibians.

Summary regarding available information and concern for endocrine disruption to aquatic species

For invertebrates adverse effects have been observed in mollusc and crustecan species. However the current data and the present level of general knowledge about the endocrine system of the tested species/ taxa do not seem sufficient to draw a firm conclusion that adverse effects as those observed or other adverse effects may be caused by Triclosan related effects on the endocrine system of these organisms.

Based on available information there is suspicion that Triclosan may cause endocrine disruption in fish (i.e for adverse effects being caused by effects which have been observed on sex hormones, vitellogenin and sperm) and amphibians (i.e. that thyroid effects, which have been observed in existing studies may lead to adverse developmental effects). The current information is however too uncertain to draw a firm conclusion. Even though estrogen- and thyroid endocrine activity has been observed in fish and amphibians the link between these effects and the observed or potential adverse effects in these taxa are currently too weak to be regarded as conclusive.

For the available studies on invertebrates, fish and amphibians described above it is currently not possible to make a final conclusion whether there is a link between the observed adverse effects of Triclosan and the observed effects on the estrogen, androgen and thyroid hormone systems in fish and amphibians. However based on the observed effects in fish and amphibians it is concluded that Triclosan has estrogenic activity in fish,

anti-androgenic effects in amphibians and fish and that it affects the thyroid hormone system in amphibians.

Appropriate further testing is requested on fish and potentially, but depending of the outcome of the requested Enhanced Developmental Neurotoxicity Study (EDNS) and any other available relevant information at a later point, also on amphibians. If the Fish sexual development test (FSDT) turns out to be negative, the eMSCA will evaluate the need for further testing for endocrine disruption in aquatic animals in relation to their development and/or disruption of their thyroid hormone system. In this context the eMSCA will consider the outcome of the requested EDNS on rats e.g. because if the test results indicate that Triclosan has indeed thyroid disrupting effects in rats, further testing of whether this is also the case for amphibians may not be needed.

In relation to further appropriate testing on fish and amphibians it has to be considered which fully validated and adopted test guidelines are currently available and what the implication of a positive / negative results would be in relation to providing a definitive conclusion or triggering further testing (c.f. OECD Guidance Document on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption (OECD, 2012).

Further invertebrate testing is currently not warranted due to a too limited general understanding of the endocrine system of invertebrates and it is at this point in time not possible to predict whether or when further endocrine concern related and targeted testing in future may be warranted on certain invertebrate taxonomic groups because this depends on future progress of science and development and validation of standard endocrine disruption related test methods on these species.

Summary of Registrant(s)'s comments and responses to comments

The Registrant(s) submitted comments and did not agree with the need for a FSDT on Triclosan (OECD TG 234) since the in vitro data are conflicting and the available in vivo data from open literature are either not indicative or correspond to toxicological effects in the fish (high mortality rate). Furthermore, he refers to additional data from a GLP guideline study on fish reproduction (US EPA guideline EPA/600/R-01/067) ([redacted]) which was not included in the registration dossier (but would be so "without undue delay") and which reveal no effects on vitellogenin levels and a number of other indicative endpoints at measured Triclosan concentrations of 3.7 – 13.5 µg/L. In addition he adds that no evidence of adverse effects of Triclosan on reproduction at concentrations that do not have general toxic effects have been observed. Furthermore no effects were seen on either fertilization rate or sex ratio in Japanese medaka (Ishibashi *et al.*, 2004; Foran *et al.*, 2000) at nominal concentrations of 200 and 100 µg/l respectively and Raut & Angus (2010) did only observe effect on sperm counts in mosquitofish at the highest test concentration of 101 µg/l where 20% mortality also occurred. Thus, there is according to the Registrant(s) not only a lack of a conclusive link to observed adverse effects, but also a lack of evidence of adverse endocrine mediated effects on fish at concentrations not causing general toxicity. Moreover, the Registrant(s) are of the opinion that general toxicity is extremely likely to occur, if the FSDT would be performed with the suggested test concentrations as 96-h LC50 values are 260 µg/L for fathead minnow (The Procter & Gamble Company, 1990) and 540 µg/L for zebrafish ([redacted]). A NOEC of 34.1 µg/L was derived in a fish early life stage test with rainbow trout (Unilever Research, 1996).

In response the eMSCA noted that the 21-day fish study (EPA/600/R-01/067) is almost equivalent to the OECD TG 229 using fathead minnow. It is well known that such at test at level 3 of the OECD conceptual framework for endocrine disrupters (OECD ED CF) cannot be used as a definitive test (i.e. such tests can only confirm endocrine activity *in vivo* but

hardly be used definitively for providing evidence of such activity providing a plausible link to adverse effects). It is also well known that the fecundity parameter of OECD TG 229 (and EPA/600/R-01/067) has a very low statistical power implying that this response variable may have provide a high relative rate of false negatives. Besides the fact that the highest test concentration was below the requested divisor of 10-12 of 96-h LC50 in this study, the Biosense vitellogenin (VTG) kit was furthermore used with a LOQ (level of quantification) of a factor 20000 above the announced LOQ which made detection of normal male vitellogenin levels impossible. In conclusion there may be several reasons relating to deficiencies of the study ([redacted]) which explains why no effect in the plasma vitellogenin concentration in males was observed after exposure to 13.5 µg/L of Triclosan. Even a 100-fold increase in serum VTG levels from background levels would not have been discovered in a study with such a poor VTG LOQ. Therefore the claim of the Registrant(s) that this study supports that no further ED related fish testing is needed is rejected. Thus the conclusion remains that further fish testing using OECD TG 234 is needed.

Based on this, testing of Triclosan in a Fish Sexual Development Test (FSDT, OECD TG 234) is required at this stage. The required test can detect estrogenic, androgenic and steroidgeneisis inhibitory activity and adverse population relevant effects (change in sex ratio) in fish.

This test, which is the only adopted OECD TG on aquatic species which, if positive, can identify endocrine disrupters in aquatic animals, is a prolonged FELS test which includes measuring of endocrine activity (change in vitellogenin (VTG) concentrations in male and female fish, changes in gonadal histology and endocrine relevant adverse effects, i.e. change of sex ratio and, if conducted in medaka, in addition secondary sex characteristics).

Existing fish toxicity test data are used as basis for establishment of appropriate exposure concentrations in the test. For acute toxicity, Oliveira *et al.* (2009) reported 96 h LC_{50} to be 420 µg/l and 340 µg/l (nominal values) in embryo- and adults zebrafish respectively. For endocrine endpoints, VTG was induced in male Japanese medaka (*Oryzias latipes*) after 21 days exposure to 20 µg/l (Ishibashi *et al.*, 2004). In Japanese medaka, 96 h LC50 has been reported from 600 µg/l to 1700 µg/l (Nassef *et al.*, 2009) in adults and from 399 µg/l to 602 µg/l in fertilized eggs and larvae (Ishibashi *et al.*, 2004). No mortality or effects on feeding were observed after 9 days exposure to 170 µg/l Triclosan (Nassef *et al.*, 2010). Raut *et al.* (2010) observed decreased sperm-counts at 101 µg/l in the western mosquitofish, *Gambusia affinis* (NOEC 57.9 µg/l). No effects of Triclosan at concentrations up to 449 ng/l were seen on newly hatched and adult fathead minnow *Pimephales promelas* during 12- and 21 days of exposure respectively (Schultz *et al.*, 2012). Based on these information sources it is concluded that appropriate exposure concentions are 15, 50 and 150 ug/L unless convincing scientific evidence can be provided which indicates that another test concentration range in zebrafish or medaka is more appropriate.

The test shall be conducted with either zebrafish or Japanese medaka. It includes measure of sex ratio (adverse effects) and vitellogenin induction in male or vitellogenin reduction in female fish (hormone activity related to the adverse effect endpoint measured). If the test is performed in medaka also secondary sex characteristics shall be included in the response parameters to report and the genetic sex should also be determined, because this endpoint may inform about any observed individual fish phenotypic sex reversal. If the test comes out positive for both endocrine activity and adverse effects, this leads in lack of counter evidence to a definitive categorisation of Triclosan as an endocrine disrupter for fish (see also level 4 of the OECD Conceptual Framework for Endocrine Distruption and the OECD Guidance Document on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption (OECD, 2012)). A change in vitellogenin concentration alone will not result in such a definitive conclusion, because also resulting adverse effects (change of sex ration)

would have to be observed. However, a change in sex ratio alone could also result in a definitive conclusion of endocrine disruption. A precondition for this is that the effect on the sex ratio is observed at concentrations not causing lethality towards mainly one of the sexes and thereby influencing the sex ratio. (c.f. further in OECD Guidance Document on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption (OECD, 2012)). A negative result in OECD TG 234 (i.e. no effects at all, or no effects on sex ratio) cannot be used conclusively for lack of endocrine disrupting properties of Triclosan in fish since the reproductive stage is not included in the FSDT test. Furthermore the FSDT test only measures certain types of sex hormone disruptive effects, namely those mentioned above causing estrogenic and androgenic and steroid genesis inhibition effects, i.e. this assay has for example not been validated for anti-androgenicity and thyroid disrupting effects.

Summary of proposals for amendment made by MSCAs, Registrant(s)'s comments to them and response to Registrant's comments

PfAs from three MSCAs and ECHA were received:

One MSCA felt that the Registrant(s) should perform a mollusc reproduction test. The MSCA stated that one example might be the draft guideline for the partial life cycle test with the freshwater gastropod *Potamopyrgus antipodarum*. The following reasoning was provided: *The available ecotoxicity data for molluscs suggest significant toxicity. The sensitivity compared to other aquatic species is not fully clear and should be investigated. We have suggested one test/species option; however the eMS may consider an alternative test/species to be more appropriate. The results of the test could be relevant to both ED and the risk assessment. Due to this, consideration should be given to tiering other aquatic vertebrate testing to follow the results of this study*.

The eMSCA responded that the sensitivity of molluscs to Triclosan seems to be high but the current knowledge about the mollusc endocrine system is generally too scarce to conclude on ED related effects and no recognized biomarkers specific to ED related effects are included in the OECD draft mollusc test guideline at the moment. The OECD draft test guidelines with either *Potamopyrgus antipodarum* or *Lymnaea stagnalis* could be used to provide information about toxicity to reproduction, but not to inform specifically about endocrine disruption, and therefore the eMSCA did not support to include molluscs in the required testing of aquatic species in relation to the concern for ED.

The Registrant(s) opposed the requirement of a mollusc reproduction test, mainly due to the absence of an internationally accepted standard guideline for long-term testing with aquatic gastropods. The Registrant(s) agreed with the eMSCA's view that further testing of Triclosan for endocrine disrupting effects in invertebrates is not recommended at this stage because it is neither needed for the PBT assessment nor for the ED assessment.

In conclusion the decision was not revised in the sections regarding the toxicity concerns for PBT or endocrine disruption for the reasons stated above.

Another MSCA made the following PfA in relation to the requested FSDT test:

We propose to delete the words "and are set at 15, 50 and 150 ug/L" and provided the following reasoning: *We do not think the exact concentrations should be specified in the Draft Decision. We have a particular concern that the top dose currently proposed is above the exposure concentration in the Fish Early Life Stage test (using rainbow trout) where 43% mortality occurred (71 µg/l). This has significant animal welfare implications for the FSDT and would potentially be a waste of fish".*

The eMSCA responded that the specific test concentrations requested have been further explained in the revised decision. The eMSCA did not support to remove all references to the recommended exposure concentrations of the requested FSDT study. Removing such a reference could lead to the conduct of an OECD TG 234 (FSDT) with test concentrations comparable to those in the 21 days OECD TG 229 like study performed with fathead minnow referred to by the Registrant(s) (Schultz *et al.*, 2012), where the maximum test concentration was only 449 ng/L Triclosan. The eMSCA regarded this study deficient in relation to the concentrations tested, i.e far too low. In relation to the comparison with toxicity levels for rainbow trout the eMSCA noted that this species is generally more sensitive to toxic effects of substances than the small fish species (medaka or zebrafish) employed in the requested OECD TG 234. Based on this the eMSCA did not find the comparison with the NOECs of the FELS employed on rainbow trout relevant in this case, because the FSDT (OECD TG 234) is requested on either the zebrafish or the Japanese medaka. Hence the originally stated test concentration range between 15 and 150 ug/L is maintained in the decision but it was added that other test concentrations may be used if scientifically justified.

A third MSCA provided the following comments and PfA:

We support the testing strategy of the eMSCA and agree that it is very useful to require optional endpoints, as it is described in the DD to assess the endocrine disruption potential of Triclosan. For performing the FSDT we recommend using the Japanese medaka, since with this species an additional endpoint (secondary sex characteristics) relevant for endocrine mediated effects can be monitored.

This endpoint and its characteristics is explicitly described for Japanese medaka in the OECD Test Guideline No. 234 ("Secondary sexual characteristics are under endocrine control in species like the Japanese medaka; therefore observations of physical appearance of the fish should if possible be made at the end of the exposure. In the Japanese medaka, the papillary formation on the posterior part of the anal fin in females is androgen sensitive. OECD TG 230 (38) provides relevant photographs of male secondary sex characteristics and androgenised females.") and is not available for the zebrafish Danio rerio. Thus, using the Japanese medaka instead of the zebrafish Danio rerio improves the explanatory power of the FSDT, especially aiming at identifying endocrine mediated effects and pathways of Triclosan.

The eMSCA responded that the secondary sex characteristics in Japanese medaka could (as described in OECD TG 234) inform about MoA of the substance. As estrogenic, androgenic and aromatase inhibition MoAs are covered in zebrafish by combining the information from the two endocrine specific endpoints vitellogenin and sex ratio, it is not recommended to exclude the possibility to conduct the test with zebrafish. It is also noted that many European laboratories are more familiar with use of zebrafish than by using the Japanese medaka, mainly due to their experience in using zebrafish in FELS testing according to OECD TG 210. For these reasons the eMSCA maintained the request to conduct the FSDT test (OECD TG 234) by use of either the zebrafish or the Japanese medaka.

If however the Japanese medaka is chosen as the species for the requested OECD TG 234 the eMSCA agreed to recommend also including the genetic sex as an endpoint because this could inform about the background for any individual phenotypic sex reversal, if such are being observed. Therefore to accommodate this, a new sentence was included in the decision.

In conclusion, a sentence was included in the decision recommending measurement of the

genetic sex in case Japanese medaka is chosen as the test species.

ECHA made the following PfA:

It is not clear from the decision what the consequences are if the FSDT test gives a negative result and whether the Registrant(s) can themself decide on the need for further testing. The second paragraph on page 43 states that "...it has later to be considered whether further testing for endocrine disruption in aquatic animals would be relevant..." and further "In this context it is also relevant to consider the outcome of the requested enhanced neurodevelopmental toxicity study on rats e.g. because if the test result here indicates that Triclosan has indeed thyroid disrupting effects in rats further testing of whether this is also the case for amphibians may not be needed." Therefore, ECHA proposes to specify in the draft decision that the need for further testing is decided in the follow-up evaluation by the eMSCAs.

New text proposed for page 43, second paragraph (of the former draft decision version / inserted by eMSCA): "Therefore, if the FSDT test turns out to be negative, the evaluating Member State will evaluate the need for further testing for endocrine disruption in aquatic animals in order to clarify the concern for endocrine disruption in aquatic animals in relation to their reproduction and/or disruption of their thyroid hormone system. In this context the evaluating Member State will consider the outcome of the requested enhanced neurodevelopmental toxicity study on rats e.g. because if the test result here indicates that Triclosan has indeed thyroid disrupting effects in rats further testing of whether this is also the case for amphibians may not be needed."

The eMSCA responded by agreeing with ECHA and the proposed text was included in a revision of the text of the decision. The text proposed was however modified in relation to the reference to the adverse effect endpoint investigated in the draft OECD LAGDA test which is developmental landmarks and not reproduction.

The Registrant(s) had the following comments to the PfAs:

The Registrant(s) do not agree with the need for this test requirement on Triclosan according to OECD TG 234 by the ECHA since the in vitro data are conflicting and the available in vivo data from open literature are either not indicative or correspond to toxicological effects in the fish (high mortality rate). Furthermore, additional data from a GLP guideline study on fish reproduction (US EPA guideline EPA/600/R-01/067) reveals no endocrine related effects in fish. This additional data, however, was not included within the initial dossier submitted to the ECHA as the study was not finalized at that time; nevertheless, this study will be part of a corresponding update dossier which will be submitted to the ECHA without undue delay.

It is stated by ECHA that "Triclosan is regarded as a suspected ED in fish because vitellogenin- and vitellogenin mRNA induction informs about estrogenicity but the links to the observed adverse effects are not fully conclusive. The biological significance of the decreased sperm counts... is not fully clear and therefore the link to an adverse effect on reproduction was missing". It has to be noted that no effects on vitellogenin levels and a number of other indicative endpoints were recorded in a 21-d fish reproduction screen with fathead minnows, a reliable GLP study, at measured Triclosan concentrations of 3.7 – 13.5 µg/L (BASF SE, 2012a). In addition and more importantly, it has to be emphasized that there is no evidence of adverse effects of Triclosan on reproduction at concentrations that do not have general toxic effects:

(1) In the 21-d fish reproduction study, fecundity and fertilisation success of fathead

minnow were not significantly affected at measured Triclosan concentrations of 3.7 – 13.5 µg/L ([redacted]*).*

(2) In the study of Ishibashi et al. *(2004), fecundity and fertilisation rate of medaka were not significantly affected at nominal concentrations of 20 – 200 µg/L*

(3) In the study of Foran et al. *(2000), sex ratio of medaka was not significantly affected at nominal Triclosan concentrations of 1 – 100 µg/L.*

(4) Effects on the sperm count in western mosquitofish were only recorded at the highest test concentration (nominal: 101 µg/L, where 20% of the fish died. At lower Triclosan concentrations (nominal: 29 and 58 µg/L), sperm count was not significantly affected (Raut & Angus, 2010).

Thus, there is not only a lack of a conclusive link to observed adverse effects, but a lack of evidence of adverse endocrine mediated effects on fish at concentrations not causing general toxicity.

The ECHA stated that an "OECD TG 234 can be used to obtain data about mode of action and its link to adverse effect, e.g. vitellogenin levels (and secondary sex characteristics in medaka) and phenotypic sex ratio" and that the "optional endpoint gonadal histopathology should be included". Most of the mentioned endpoints, namely vitellogenin levels, secondary sexual characteristics and, to a limited extent, gonad histology, have already been evaluated in the 21-d fish reproduction study ([redacted]*), in which no significant effects on indicative and apical endpoints were recorded.*

A fish sexual development test would allow to additionally evaluate the endpoint sex ratio. As outlined in OECD (2012), performing a TG 234 test is particularly relevant for test substances suspected to act primarily on the sexual development rather than on the reproductive phase. However, there is no indication of a particular effect of Triclosan on sex ratio: as mentioned above Foran et al. *(2000) did not record any significant effect on sex ratio of medaka at nominal Triclosan concentrations of 1 – 100 µg/L.*

<u>With respect to the conclusion in fish:</u> The requirement of a fish sexual development test (FSDT, OECD TG 234) is not considered as necessary due to the following reasons: Based on the absence of significant adverse endocrine mediated effects on fish at concentrations not causing general toxicity as recorded in the 21-d fish reproduction study ([redacted]*) as well as in the studies by Foran* et al. *(2000) and Ishibashi* et al. *(2004) it can be concluded that Triclosan is not sufficiently potent to produce endocrine-mediated adverse effects in vivo. Hence, there is no evidence of serious adverse effects on fish that are linked to an endocrine mode of action. In view of this fact and the lack of indication that Triclosan primarily acts on sexual development, there is no convincing reason for performing a fish sexual development test.*

As outlined above and with specific comment to the suggested test concentrations, there is no convincing rationale for performing an OECD TG 234 test. Moreover, general toxicity is extremely likely to occur, if the FSDT would be performed with the suggested test concentrations: 96-h LC_{50} values are 260 µg/L for fathead minnow (The Procter & Gamble Company, 1990) and 540 µg/L for zebrafish ([redacted]*). A NOEC of 34.1 µg/L was derived in a fish early life stage test with rainbow trout (Unilever Research, 1996). In medaka, 20% mortality was observed during a 14-d exposure to 100 µg/L (Foran et al. 2000).*

Regarding the ECHA request for another OECD 229, it can be stated that results of a 21-d

fish reproduction study according to US EPA guideline EPA/600/R-01/067 (US EPA, 2002), which is very similar to OECD TG 229, are available ([redacted]). This test allows detecting effects of estrogens, anti-estrogens, androgens, anti-androgens, aromatisable androgens and aromatase inhibitors. It also provides information on adverse effects on reproduction that could be used in environmental risk assessment (OECD, 2012). Only if test conditions are sub-optimal, statistical power is low (OECD, 2012). However, the available 21-d fish reproduction study provides clear evidence of a lack of effects on fecundity and fertility. Variation between replicates is low.

The Registrant(s) also provided comments to the PfA requesting the FSDT conducted on medaka and not zebra fish by providing the following remarks:

The Registrant(s) agree with the proposed fish test but doesn´t see a significant difference in the fish species proposed for the testing. Both medaka and zebrafish are well established at various laboratories which reduces potential negative impacts on the study performance due to poor handling, although there might be a preference in one of the species in specific labs. Secondary sex characteristics are clearly present in Japanese medaka while they are only subtle in zebrafish. Nevertheless, it has been shown in scientific literature that secondary sex characteristics are less sensitive than other biomarkers. Dang et al. *(2011) [35] compared 142 data sets for 21-day fish assays (OECD TG 229) and 38 data sets for the fish sexual development test (FSDT), encompassing 62 chemicals with different modes of action (MOAs). The authors concluded that: (1) vitellogenin (VTG), fecundity and gonad histology are the most sensitive endpoints for fathead minnow, medaka and zebrafish in 21-day fish assays. In contrast, secondary sex characteristics are a less sensitive endpoint and is likely inadequate to detect all known MOAs. Regarding the results from the review by Dang* et al. *(2011) [35], a negative impact on the performance of the FSDT with zebrafish instead of medaka is not to be expected.*

In fact, no scientific sound rationale is given which votes for either zebra fish or medaka, beyond the availability of the test fish at the corresponding testing lab. Again, the Registrant(s) recommends that the test species should not be specified to either zebrafish or medaka."

Finally the Registrant(s) commented on ECHAs PfA regarding the text on the draft decision concerning the need for further amphibian testing when follow up is being considered after the enhanced neutrodevelopmental toxicity tests has been conducted and evaluated and if the FSDT test turns out negative, i.e. that only by considering the results of the former test it can be decided whether actually any further follow-up testing in amphibians are warranted relating to the concern for thyroid effects. Based on this the Registrant(s) proposed not to conduct the FSDT test before the Enhanced Developmental Neurotoxicity Study has been completed and evaluated.

The eMSCA responded by acknowledging the current support of the Registrant(s) to conduct the requested FSDT test.

The comments to the first mentioned PfA relating to the need for a FSDT test were however repeating the earlier provided comments of the Registrant(s) which have been fully addressed above and hence no further response is needed.

The decision has not been revised due to the second mentioned PfA concerning requesting the FSDT test to be performed in medaka and not providing the option to alternatively to select the zebrafish and this conclusion is hence in accordance with the conclusion of the Registrant(s) on this issue.

Finally in relation to ECHAs PfA the eMSCA was of the opinion that the Registrant(s) have misunderstood the content as the Registrant(s) in their comments refers to "testing" and not "further testing". ECHA proposes to consider in the follow-up the need for further testing in relation to potential thyroid effects. ECHA does not propose to avoid requesting the FSDT test now. Hence the decision was not revised based on this comment of the Registrant(s).

Finally the Registrant(s) have also provided a range of specific comments to other parts of the draft decision relating to *in vitro* data on endocrine related tests/endpoints. These comments and the responses to them by the eMSCA are addressed in the attached Annex 2.

Conclusion

Based on the information above, a Fish Sexual Development Test is requested (FSDT, OECD TG 234). The test shall include a test concentration range of 15, 50 and 150 ug/L in medaka or zebrafish unless scientific evidence justifies another testing range. In case Japanese medaka is chosen as the test species, determination of the genetic sex is recommended.

If the FSDT test turns out to be negative, the eMSCA will evaluate the need for further testing for endocrine disruption in aquatic animals in order to clarify the concern for endocrine disruption in aquatic animals in relation to their development and/or disruption of their thyroid hormone system. In this context the eMSCA will consider the outcome of the requested Enhanced Neurodevelopmental Toxicity study on rats e.g. because if the test result here indicates that Triclosan has indeed thyroid disrupting effects in rats further testing of whether this is also the case for amphibians may not be needed.

III.III Concerns regarding cardiotoxicity

4. Cardiotoxicity

Initial requirement

This endpoint was not part of the initial draft decision.

Summary of proposals for amendment made by MSCAs, Registrant(s)'s comments on them and responses to Registrant's comments

During the consultation period, the eMSCA received a PfA from one MSCA, to include a new identified endpoint of concern in the substance evaluation. This MSCA referred to a recent published study of Cherednichenko et al (2012) who reported that Triclosan impairs excitation-contraction coupling (ECC) of both cardiac and skeletal muscle in vitro and in vivo by influencing ECC and Ca^{2+} dynamics in striated muscle.

Impairment on excitation-contraction coupling and Ca^{2+} dynamics in striated muscle by Triclosan was observed in the study by Cherednichenko *et al.* (2012). According to this study, a cardiotoxic effect on humans is suspected.

The Registrant(s) commented on the PfA by stating that *"An MS also commented on a study by Cherednichenko* et al. *[21] who found effects on muscle function following intraperitoneal administration of Triclosan to mice. The doses which are presented in the article are very much higher than those to which a typical consumer would be exposed. The European Commission's independent safety experts SCCS recommend a safe exposure level of Triclosan in common use cosmetic products of 0.1077 mg/kg body weight/day. In the study a level of 6.25 to 25 mg/kg was used, which is more than 60 times higher than the*

established safe exposure level. In the cited study, mice were exposed intraperitoneally (i.p.), i.e. into the abdominal cavity. This route of exposure is suitable to maximize toxic effects but has little to no relevance to the human exposure situation. Humans are exposed to Triclosan primarily via the dermal, and to a lesser extent via the oral route. i.p. administration circumvents barriers and mechanisms of detoxification. The skin represents a good barrier, leaving ca. 90% of the Triclosan applied unabsorbed. For instance, dermal absorption (as calculated from the amounts of Triclosan recovered in the dermis and epidermis layers) was 12%, 7.7% and 7.2% for a dishwashing (0.2%), deodorant (0.2%), and soap (0.02%, 10 min incubation)) formulation, respectively [22-24]. In addition, there is a pronounced first pass effect [25, 26] which explains why almost no unconjugated Triclosan is detected in animal or human studies with relevant routes of exposure. The authors of the cited study claim that in mice, plasma Triclosan concentrations parallel those in human studies with oral exposure. Blood serum levels as determined by Cherednichenko et al. were in the low µM range (ca. 0.14, 0.23 and 0.31 µM for doses of 6.25, 12.5 and 25 mg/kg, respectively). In oral studies in the mouse with single administration [27], concentrations were a factor of 170 higher at the 30 min reading time at ca. 27 µM (parent equivalents) for the 2 mg/kg dose group. In the 200 mg/kg dose group, blood levels were as high as ~ 600 µM at the 30 min reading time. In human studies with oral administration of Triclosan capsules, a steady state concentration of ca. 1.40 µM was achieved with a dose of 15 mg/day (0.25 mg/kg for a 60 kg person). This total Triclosan concentration consisted of the Triclosan sulphate (1.35 µM or 96%), Triclosan glucuronide (0.048 µM or 3.4%) and parent Triclosan (0.005 µM or 0.36%). Under normal use conditions during twice daily tooth brushing, total Triclosan steady state levels reached 0.073 µM with (except for one subject) no detectable amounts of parent Triclosan [28]. Hence, the Triclosan from human studies was almost completely metabolized whereas in the study by Cherednichenko et al., *an amount of unmetabolized Triclosan similar to the amount of total Triclosan in some human studies was detected rendering the comparison irrelevant. Moreover, adverse effects related to exposure to free Triclosan as in the cited study with i.p. administration are likely irrelevant to other routes of exposure. The authors fail to explain that, although their lowest dose group was three times as high as the dose used in the oral mouse study, plasma levels were significantly (170-fold) lower with apparently only unconjugated Triclosan present in the blood plasma.*
A long term mouse study is available that was conducted with Triclosan up to 200 mg/kg daily exposure [17], i.e. about 10-fold the amount used in the cited study. Neither was the heart a target organ after 18 month exposure nor where there any morphological or histopathological (tissue) changes in the heart that would indicate an adverse effect on the organ. Neurotoxic effects were not reported in any species tested to date with oral, dermal or inhalation exposure to Triclosan. A two week neurotoxicity study in rats [29] also failed show an effect at concentrations 4 times (100 mg/kg) those used by Cherednichenko et al. *and revealed no specific neurotoxicity at concentrations up to 300 mg/kg. In addition, no pathological chances were found in the brain and in the peripheral nerves.*

A tolerance and pharmacokinetic study in 20 human volunteers was conducted with escalating daily doses of Triclosan in capsules (0-30 mg/capsule) [26]. Single dose and repeated doses were given depending on the study phase (up to 30 days). Cardiac examinations (ECG), lung function and neurological examinations revealed no overt effects. The study confirmed that a pronounced first-pass-effect is responsible for an almost complete conversion of Triclosan to its metabolites, with only trace amounts of the parent detectable in blood (ca. 5 nM and 4 nM parent Triclosan under steady state and single dose conditions, respectively). It can be concluded that doses that lead to a maximum steady-state concentration of ca. 2 µM Triclosan (combined glucuronide, sulfate and parent) over 30 days did not adversely target the heart".

The eMSCA concluded that the information required is appropriate as no information on this

issue is available in the submitted dossier. Therefore the potential of Triclosan to produce cardiotoxicity shall be assessed by the Registrant(s). To do this, all available information on the effects on the cardiovascular system on laboratory animals and humans shall be provided and the registration dossier updated, including provision of available information regarding blood pressure, heart rate and electrocardiogram. All the available information on human vigilance shall also be provided.

The eMSCA will evaluate whether further targeted follow up studies are required when it has received and evaluated the requested information.

III.IV Concerns regarding exposure

5. Further information on the environmental emission scenario 'Wide dispersive indoor use of reactive substances in open systems'

Initial requirement

This endpoint was not part of the initial draft decision.

Summary of proposals for amendments made by MSCAs, Registrant(s)'s comments on them and response to Registrant(s)'s comments

One PfA was received on the calculations used to derive the PEC for end use in the consumer use lifecycle stage in section 9.4 of the Chemical Safety Report. It was noted that the required operational conditions and assumptions were not clearly specified and do not allow to reproduce the exposure calculations. Most notably, information on the concentration in consumer products and assumed level of biodegradation was missing. In addition, after updating the exposure calculations, it was suggested that the Registrant(s) should consider whether additional risk management measures are necessary to ensure environmental concentrations do not exceed the PNEC.

The Registrant(s) noted that the use of Triclosan in cosmetics and personal care products is defined according to the Cosmetic Regulation (EC) No 1223/2009 and the use concentration is only allowable to a maximum of 0.3%. Professional uses of and consumer use as surface disinfectants are regulated by the Biocidal Products Regulation (EU) No 528/2012 in Europe.

The eMSCA concluded that, in order to clarify the environmental emission scenario in the dossier termed 'Wide dispersive indoor use of reactive substances in open systems', the following information is required from the Registrant(s):

The Registrant(s) shall provide the calculations used to derive the PECs for 'Wide dispersive indoor use of reactive substances in open systems', in section 9.4 of the CSR. They shall also provide justification for the parameters used to determine releases to environmental compartments, specifically:

- Describe which activities are covered under this exposure scenario that lead to wide dispersive use of Triclosan
- Relevant tonnage for consumer end use (total product)
- Number of release days (days/year),
- Assumed percentage of Triclosan in the formulated consumer products
- Release fraction to air, wastewater and soil (%), since the applied Environmental Release Class 8b appears to lead to unrealistically low release fractions
- Fraction of main source (%),
- Sewage treatment plant flow (m3/d),

- Flow of receiving water (m3/d),
- Dilution factor (-).
- Details of the assumed biodegradation rate for simulating fate in the WWTP, based on the updated dossier with regard to biodegradation
- Resulting PEC values at local and regional scale: indicate the software and version number used to estimate local and regional PEC values (e.g. EUSES, ECETOC TRA, etc.).

The Registrant(s) shall take into account that where the available monitoring data in the literature indicate higher exposure concentrations than calculated PECs (i.e., the outcome of item 5.1), additional risk management measures may be necessary to ensure environmental concentrations do not exceed the PNEC. These shall then be reported in the CSR as part of the exposure scenarios for the respective end uses, section 9.4 of the CSR.

Conclusion

The exposure scenario in Section 9.4 of the Chemical Safety Report shall be updated including the information requested in Section II.IV.

IV. Adequate identification of the composition of the tested material

The substance identity information submitted in the registration dossiers has not been checked for compliance with the substance identity requirements set out in Section 2 of Annex VI of the REACH Regulation.

The Registrant(s) are reminded of his responsibility to ensure that his registration covers one substance only and that the substance is correctly identified in accordance with Annex VI, Section 2 of the REACH Regulation.

In carrying out the studies required by the present decision it is important to ensure that the particular sample of substance tested is appropriate to assess the properties of the registered substance, taking into account any variation in the composition of the technical grade of the substance as actually manufactured. If the registration of the substance covers different grades, the sample used for the new studies must be suitable to assess these.

Furthermore, there must be adequate information on substance identity for the sample tested and the grade(s) registered to enable the relevance of the studies to be assessed.

V. General requirements regarding Good Laboratory Practice

ECHA always reminds Registrant(s) of the requirements of Article 13(4) of the REACH Regulation that ecotoxicological and toxicological tests and analyses shall be carried out in compliance with the principles of good laboratory practice (GLP). National authorities monitoring GLP maintain lists of test facilities indicating the relevant areas of expertise of each facility.

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.

EUROPEAN CHEMICALS AGENCY

[redacted]

Jukka Malm
Deputy Executive Director

Enclosures: Annex 1
Annex 2
Annex 3

Annex 1

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Annex 2

Registrant(s)'s comments and eMSCA responses on *in vitro* data regarding ED related data.

(please note that the page numbers refer to the original draft decision as commented on by the Registrant(s))

Comment 1 by the Registrant(s):

Estrogenicity (E) and Androgenicity (A)

In vitro data (E+A)

On p. 9-10 of the ECHA draft decision, it is stated that it was shown in a study of Gee et al. (2008) that "Triclosan was capable of producing both estrogenic and androgenic effects". Please note that Gee et al. (2008) performed competitive binding assays with the human estrogen receptor (hER) and the human androgen receptor (hAR). The results of these assays demonstrate binding of Triclosan to the hER and the hAR rather than estrogenic or androgenic effects.

Response 1 by the eMSCA:

In the ECHA draft decision the study design used in the Gee et al (2008) study was briefly explained, as it was stated that Triclosan displaced estradiol from estrogen receptors and inhibited testosterone from binding to the androgen receptor, so the reader was informed of what the study showed, but the eMSCA agrees that the results demonstrate binding of Triclosan to the steroid hormone receptors rather that estrogenic and androgenic effects *per see.*

Comment 2 by the Registrant(s):

Furthermore, it is stated in the ECHA draft decision that Christen et al. (2010) found "Triclosan to act as both a partial AR agonist and as an AR antagonist". It should be noted that according to Christen et al. (2010) Triclosan alone has a low androgenic activity. In the presence of dihydrotestosterone,Triclosan increased the androgenic effect of dihydrotestosterone. However, in a previous study with the same reporter gene assay Triclosan was found to be an AR antagonist (Tamura et al. 2006). Due to these conflicting results, which are unfortunately not discussed by Christen et al. (2010), these findings should be considered with care.

Response 2 by the eMSCA

This data is however of limited relevance for the assessment of Triclosan as a potential endocrine disrupter.

Endocrine Disruption, Thyroid (T)

In vitro data (T)

Comment 3 by the Registrant(s)

Triclosan has shown to alter thyroid homeostasis in vitro (Veldhoen et al., 2006)". Veldhoen

et al. (2006) studied the expression of four thyroid hormone associated genes - thyroid hormone receptors α and β, the basic transcription element binding protein and the proliferating nuclear cell antigen - in the presence and in the absence of 3,5,3'-triiodothyronine (T3). It should be noted that effects were only observed in the presence of T3. In the absence of T3, Triclosan had no effect on the expression of the above-mentioned genes.

Response 3 by the eMSCA

Since T3 would always be present in an *in vivo* situation, this observation does not make these results any less relevant.

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Annex 3

Reference list provided by the Registrant(s) in the document containing comments to the PfAs.

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