ECHA
EUROPEAN CHEMICALS AGENCY

Decision number: TPE-D-0000002203-88-05/F Helsinki, 2 July 2012

DECISION ON A TESTING PROPOSAL SET OUT IN A REGISTRATION PURSUANT TO ARTICLE 40(3) OF REGULATION (EC) NO 1907/2006

For Coconut oil, reaction products with polyethylene glycol and trimethylolpropane, CAS No. [redacted] (EC No. 640-964-5), registration number: [redacted]

Addressee: [redacted]

The European Chemicals Agency (ECHA) has taken the following decision in accordance with the procedure set out in Articles 50 and 51 of Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH Regulation).

I. Procedure

Pursuant to Article 40(1) of the REACH Regulation, ECHA has examined testing proposals set out in the registration dossier for Coconut oil, reaction products with polyethylene glycol and trimethylolpropane, CAS No. [redacted] (EC No. 640-964-5), submitted by [redacted] (Registrant), latest submission number [redacted], for 100 to 1000 tonnes per year.

In accordance with Articles 10(a)(ix) and 12(1)(d) of the REACH Regulation, the Registrant submitted the following testing proposals as part of the registration dossier to fulfil the information requirements set out in Annex IX:

1. Mammalian erythrocyte micronucleus test, administration route not specified (OECD Guideline 474);
2. Repeated dose 90-day oral toxicity in rodents, oral (OECD Guideline 408);
3. Pre-natal developmental toxicity study (OECD Guideline 414); and
4. *Daphnia magna* reproduction test (OECD Guideline 211);

The examination of the testing proposals was initiated upon the date when receipt of the complete registration dossier was confirmed on 19 July 2011.

ECHA opened a third party consultation for the testing proposals including testing on vertebrate animals that was held from 15 September 2011 until 31 October 2011. ECHA did receive information from third parties (see section III below).

On 5 January 2012 ECHA sent the draft decision to the Registrant and invited him to provide comments within 30 days of the receipt of the draft decision.

On 11 January 2012 ECHA received comments from the Registrant. ECHA considered the Registrant's comments received and did amend the draft decision.

On 2 March 2012 ECHA notified the Competent Authorities of the Member States of its draft decision and invited them pursuant to Article 51(1) of the REACH Regulation to submit proposals to amend the draft decision within 30 days of the receipt of the notification. Subsequently, one Competent Authority of a Member State submitted proposals for amendment to the draft decision. ECHA reviewed the proposals for amendment received and did not amend the draft decision.

On 4 April 2012 ECHA notified the Registrant of proposals for amendment to the draft decision and invited him pursuant to Article 51(5) of the REACH Regulation to provide comments on those proposals for amendment within 30 days of the receipt of the notification.

On 16 April 2012 ECHA referred the draft decision to the Member State Committee.

On 23 April 2012 April the Registrant provided comments on the proposed amendments. The Member State Committee took the comments of the Registrant into account.

A unanimous agreement of the Member State Committee on the draft decision was reached on 21 May 2012 in a written procedure launched on 10 May 2012.

This decision does not imply that the information provided by the Registrant in his registration dossier is in compliance with the requirements of the REACH Regulation. The decision does not prevent ECHA to initiate a compliance check on the present dossier at a later stage.

II. Testing required

Pursuant to Article 40(3)(a) of the REACH Regulation, the Registrant shall carry out the following proposed tests using the indicated test methods and the registered substance subject to the present decision:

1. Mutagenicity – *in vivo* mammalian erythrocyte micronucleus test, oral route (Annex IX, 8.4., test method: EU B.12/OECD 474);
2. Sub-chronic toxicity study (90-day) in rats, oral route (Annex IX, 8.6.2., test method: EU B.26/OECD 408);
3. Pre-natal developmental toxicity study in rats, oral route (Annex IX, 8.7.2., test method: EU B.31/OECD 414) and
4. *Daphnia magna* Long term toxicity to invertebrates (Annex IX, 9.1.5., test method: EU C.20/OECD 211).

The Registrant shall determine the appropriate order of the studies 1-3 taking into account the possible outcomes and considering the possibilities for adaptations of the standard information requirements according to column 1 or 2 provisions of the relevant Annexes of the REACH Regulation.

Pursuant to Articles 40(4) and 22 of the REACH Regulation, the Registrant shall submit to ECHA by **2 July 2014** an update of the registration dossier containing the information required by this decision.

At any time, the Registrant shall take into account that there may be an obligation to make every effort to agree on sharing of information and costs with other Registrants.

III. Statement of reasons

The decision of ECHA is based on the examination of the testing proposal submitted by the Registrant for the registered substance and scientific information submitted by third parties.

a) Examination of the testing proposal

1. Mutagenicity – *in vivo* mammalian erythrocyte micronucleus test

Pursuant to Article 40(3)(a) of the REACH Regulation, ECHA may require the Registrant to carry out the proposed test.

The Registrant proposes to conduct an *in vivo* micronucleus test following EU test method B.12.

The Registrant has assessed genetic toxicity of Coconut oil, reaction products with polyethylene glycol and trimethylolpropane in several in vitro systems. *In vitro* studies in bacteria (*Salmonella typhimurium* strains TA 98, TA 100, TA 1535, and TA 1537) and mouse lymphoma L5178Y cells have demonstrated no genetic toxicity of Coconut oil, reaction products with polyethylene glycol and trimethylolpropane when tested in the presence or absence of exogenous metabolic activation (S9). A Chromosomal aberration test in Chinese Hamster Ovary cells demonstrated weak effects only in the presence of exogenous metabolic activation. Therefore, a micronucleus test *in vivo* was proposed by the Registrant.

If there is a positive result in any of the *in vitro* genotoxicity studies in Annex VII or VIII and there are no results available from an *in vivo* study already, an appropriate *in vivo* somatic cell genotoxicity study shall be proposed by the Registrant as laid down in Annex IX, section 8.4. of the REACH Regulation. The information on this endpoint is not available for the registered substance but needs to be present in the technical dossier to meet the information requirements. Consequently there is an information gap and it is necessary to generate the data for this endpoint.

The Registrant has not proposed an administration route. According to the EU test method B.12. the test substance is usually administered orally. In the light of the physico-chemical properties and the Registrant's assessment on toxicokinetics on the registered substance using read-across for some of the constituents in the substance of unknown or variable composition, complex reaction products or biological materials (UVCB substance) such as the registered substance subject to the present decision and the information provided on the uses and human exposure, ECHA considers that testing by the oral route is appropriate.

The Registrant did not specify the species to be tested. According to the test method EU B.12/OECD 474 mice or rats are recommended if bone marrow is used. When peripheral blood is used, mice are recommended. However, any appropriate mammalian species may be used provided it is a species in which the spleen does not remove micronucleated erythrocytes or a species which has shown an adequate sensitivity to detect substances that cause structural or numerical chromosome aberrations. ECHA considers any of these species options as being appropriate. However, the Registrant should consider the toxicokinetic properties or any other information concerning the registered substance which may have an impact on the selection of the target tissue and thereby the test species.

The Registrant expressed consent to the third party proposal to combine the *in vivo* micronucleus assay with the 90-day study protocol. ECHA does not consider this as

appropriate (see below b) p. 6 consideration of third party information 3).

Therefore, pursuant to Article 40(3)(a) of the REACH Regulation, the Registrant is required to carry out the proposed test: *in vivo* mammalian erythrocyte micronucleus test, oral route (Annex IX, 8.4., test method: EU B.12/OECD 474) using the registered substance Coconut oil, reaction products with polyethylene glycol and trimethylolpropane.

2. Repeated dose toxicity

Pursuant to Article 40(3)(a) of the REACH Regulation, ECHA may require the Registrant to carry out the proposed test.

The Registrant proposed sub-chronic toxicity study (90 day) toxicity in rodents by oral administration following protocol OECD 408.

A sub-chronic toxicity study (90 day) is a standard information requirement as laid down in Annex IX, section 8.6.2. of the REACH Regulation. The information on this endpoint is not available for the registered substance but needs to be present in the technical dossier to meet the information requirements. Consequently there is an information gap and it is necessary to generate the data for this endpoint.

In the light of the physico-chemical properties of the registered substance concerned by the present decision and the Registrant's toxicokinetic assessment using read-across for some of the constituents in the registered UVCB substance subject to the present decision and the information provided on the uses and human exposure, ECHA considers that testing by the oral route is appropriate.

The Registrant did not specify the species to be tested. According to the test method EU B.26/OECD 408 the rat is the preferred rodent species. ECHA considers this species as being appropriate.

The Registrant expressed consent to the third party proposal to combine the *in vivo* micronucleus assay with the 90-day study protocol. ECHA does not consider this as appropriate (see below b) p. 7 consideration of third party information 3).

Therefore, pursuant to Article 40(3)(a) of the REACH Regulation, the Registrant is required to carry out the proposed test: Sub-chronic toxicity study (90-day) in rats, oral route (test method: EU B.26/OECD 408) using the registered substance Coconut oil, reaction products with polyethylene glycol and trimethylolpropane.

3. Pre-natal developmental toxicity

Pursuant to Article 40(3)(a) of the REACH Regulation, ECHA may require the Registrant to carry out the proposed test.

The Registrant has proposed to conduct a pre-natal developmental toxicity study (OECD Guideline 414).

A pre-natal developmental toxicity study for a first species is a standard information requirement as laid down in Annex IX, section 8.7.2. of the REACH Regulation. The information on this endpoint is not available for the registered substance but needs to be present in the technical dossier to meet the information requirements. Consequently there

is an information gap and it is necessary to generate the data for this endpoint.

The Registrant proposes to use oral administration route but did not specify the species. According to the test method EU B.31/OECD 414, the rat is the preferred rodent species, the rabbit the preferred non-rodent species and the test substance is usually administered orally. ECHA considers these default parameters appropriate and testing should be performed by the oral route with the rat as a first species to be used.

Therefore, pursuant to Article 40(3)(a) of the REACH Regulation, the Registrant is required to carry out the proposed test: Pre-natal developmental toxicity study in rats, oral route (test method: EU B.31/OECD 414) using the registered substance Coconut oil, reaction products with polyethylene glycol and trimethylolpropane.

4. Long term toxicity to invertebrates

Pursuant to Article 40(3)(a) of the REACH Regulation, ECHA may require the Registrant to carry out the proposed test.

An experimental study is proposed for long-term toxicity to *Daphnia magna* following OECD Guideline 211. In the chemical safety report (CSR), the Registrant gives the following justification for testing in *Daphnia magna*: "The short-term toxicity of Coconut oil, reaction products with polyethylene glycol and trimethylolpropane was much higher for Daphnia magna than for fishes. Therefore an experimental study is planned for long-term toxicity to *Daphnia magna*."

A *Daphnia magna* reproduction test is a standard information requirement as laid down in Annex IX, section 9.1.5. of the REACH Regulation. The information on this endpoint is not available for the registered substance but needs to be present in the technical dossier to meet the information requirements. Furthermore, findings of the short-term studies on *Daphnia* indicate the need for further (long-term) testing as indicated in the CSR. Consequently there is an information gap and it is necessary to generate the data for this endpoint. It is also the opinion of ECHA that based on the acute aquatic toxicity information on *Daphnia* the long-term toxicity test should be conducted in the same species.

ECHA reminds the Registrant of the need for analytical monitoring in the long-term studies to be conducted. Furthermore, the Registrant shall follow an optimal procedure for aquatic testing of poorly water soluble/hydrophobic substances as described in in the EU C.20/OECD 211 Guideline and the OECD Guidance document No. 23 on aquatic toxicity testing of difficult substances and mixtures (ENV/JM/MONO(2000)6). Account of this should also be taken when interpreting the results of the test.

Therefore, pursuant to Article 40(3)(a) of the REACH Regulation, the Registrant is required to carry out the proposed test: *Daphnia magna* reproduction test (Annex IX, 9.1.5., test method: EU C.20/OECD 211) using the registered substance Coconut oil, reaction products with polyethylene glycol and trimethylolpropane.

b) Consideration of third party information

ECHA received third party information concerning the testing proposal during the public consultation. For the reasons explained further below the information provided by third parties is not sufficient to fulfil this information requirement.

Third party information 1 (Mutagenicity) and 2 (Repeated dose toxicity and prenatal developmental toxicity)

A third party suggested to disregard the positive result of the in vitro mammalian chromosome aberration test because it considers it is due to cytotoxicity and a p53 gene mutation.
The third party has also proposed a weight of evidence approach for ECHA to consider before further tests on animals are requested. As part of this approach, the third party refers to US EPA polyol esters category by using the read-across substance Polyol ester, carboxylic acids C5-9, tetraesters with pentaerythritol (CAS No. 67762-53-2). In addition, the third party refers to three surrogate polyol esters (CAS No. 189120-64-7; 180788-27-6; and PE esters with isooctanoic and C8-10 fatty acid. The third party states that there is sufficient weight of evidence from *in vitro* and *in vivo* studies in polyalcohols which cannot substantiate clastogenic properties on this group of substances.

More specifically the third party refers to the 28-day study results of trimethylol propane ester of heptanoic and octanoic acid (CAS No. 189120-64-7) and a 13 week study result of pentaerythriol tetraester of C5-9 (CAS No. 67762-53-2).

Furthermore, the third party refers to the prenatal developmental toxicity evaluation of decanoic acid, ester with 2-ethyl-2(hydroxy methyl)-1,3-propanediol octanoate (CAS No. 11138-60-6) and pentaerythriol tetraester of C5-9 (CAS No. 67762-53-2) of the OECD SIDS polyol alcohol dossier.

The third party states that it was concluded in the test plan that not the ester compounds but ester hydrolysates with free polyol alcohols could exhibit a low developmental toxicity potential according to the OECD SIDS of polyol alcohols.

ECHA considers that although the information provided by the third party might be scientifically valid, it is still not sufficient for ECHA to conclude that the conditions of Annex XI of the REACH Regulation are met.

More specifically, the registered substance is a derivative of a vegetable oil and clearly identified as UVCB substance. It consists primarily of C8-C50 fatty acid esters of polyethylene glycol, trimethylolpropane and glycerol. The analytical data indicates a presence of a broad range of component substances each of which is present at low concentrations. The most significant component of the substance is C12 monoglyceride at a concentration of [redacted] while other components typically have a concentration of [redacted] with over 50 substances present at very low concentrations in the product.

The third party proposes that the SIDS dossiers for dipentaerythritol, pentaerythritol, trimethylolpropane, or US EPA dossiers for glycol esters should be used by a weight of evidence approach for the repeated dose toxicity of the substance subject to the present decision. Furthermore the third party proposes to use as weight of evidence for mutagenicity the substances polyol ester, carboxylic acids C5-9, tetraesters with pentaerythritol (CAS No. 67762-53-2) and polyol esters (CAS No. 189120-64-7; 180788-27-6; and PE esters with isooctanoic and C8-10 fatty acid.

ECHA cannot assume that the substance has or has not a particular dangerous property of any single substances or categories of substances. Moreover, due to the complex nature of the registered substance structural similarity could not be sufficiently demonstrated.

Even if ECHA cannot draw a definitive conclusion from the information provided it acknowledges that the Registrant may himself supplement under its own responsibility the argumentation and information provided by the third party in order to make use of adaptation possibilities. This would require that the Registrant demonstrates a sufficient justification from several independent sources of information leading to the assumption/conclusion that a substance has or has not particular dangerous properties, according to the criteria laid down in Annex XI of the REACH Regulation.

Third party information 3 (Combining the repeated dose toxicity and the *in vivo* micronucleus test protocols)

In order to reduce animal numbers, the third party has proposed combining the *in vivo* micronucleus test (OECD 474) and the subchronic toxicity study (OECD 408) and refers to the Scientific opinion on genotoxicity testing strategies applicable to food and feed safety assessment (EFSA Scientific Committee, 2011) and Draft Consensus Guideline "Guidance on Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use S2(R1) (ICH, 6 March 2008) and Guidance on Information Requirements, R.7.a, Mutagenicity and Carcinogenicity, p377 (ECHA, May 2008).

In its comments, the Registrant expressed consent to the proposed combined study protocol.

The above mentioned guidance documents give general recommendations on genotoxicity testing strategies. However, in ECHA's opinion they are not study guidelines and as such they do not give sufficiently detailed instructions on how to perform such a combined 90-day subchronic study and an *in vivo* micronucleus test. Furthermore, in ECHA's opinion in combining these protocols there are multiple factors, which are likely to confound the result and complicate the study design, such as, the maximum tolerated dose of the 90-day study may be too low for the purposes of the micronucleus study; the impact of animal aging when comparing to historical controls; and the fact mice may need to be used instead of rats if peripheral blood samples are to be collected whereas rat is the preferred species for a 90-day study. In addition, currently, there is no internationally accepted guideline for a combined 90-day subchronic study and an *in vivo* micronucleus test.

Therefore, in the absence of a proper test guideline or further details on the experimental protocol from the Registrant ECHA does not consider there is sufficient scientifically relevant information to reject the two tests proposed by the Registrant and require a combined assay instead.

IV. General requirements for the generation of information and Good Laboratory Practice

ECHA always reminds registrants 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.

According to Article 13(3) of the REACH Regulation, tests that are required to generate information on intrinsic properties of substances shall be conducted in accordance with the test methods laid down in a Commission Regulation or in accordance with other international test methods recognised by the Commission or the European Chemicals Agency as being appropriate. Thus, the Registrant shall refer to Commission Regulation

(EC) No 440/2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 as adapted to technical progress or to other international test methods recognised as being appropriate and use the applicable test methods to generate the information on the endpoints indicated above.

V. Information on right to appeal

An appeal may be brought against this decision to the Board of Appeal of ECHA under Article 51(8) of the REACH Regulation. Such 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/appeals/app_procedure_en.asp. The notice of appeal will be deemed to be filed only when the appeal fee has been paid.

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