EUROPEAN CHEMICALS AGENCY

Helsinki, 30 March 2017

Addressee: [redacted]

Decision number: CCH-D-2114355769-31-01/F
Substance name: vinyl acetate
EC number: 203-545-4
CAS number: 108-05-4
Registration number: [redacted]
Submission number: [redacted]
Submission date: 02.07.2014
Registered tonnage band: 1000+T

DECISION ON A COMPLIANCE CHECK

Based on Article 41 of Regulation (EC) No 1907/2006 (the 'REACH Regulation'), ECHA requests you to submit information on

1. **In vivo mammalian alkaline comet assay (Annex X, Section 8.4., column 2; test method: OECD TG 489) in rats, oral route, by gavage, on the following tissues: liver, glandular stomach and duodenum with the registered substance; two sets of slides shall be prepared and analysed, one set submitted to standard experimental conditions and one set submitted to modified experimental conditions that enable the detection of DNA crosslinks. The modified protocol shall include treatment by MMS (methyl methanesulfonate) or by ionising irradiation (according to e.g. references 36-39 in the TG 489 or Pant et al 2015), as well as a specific positive control group of animals. The test material used should be freshly prepared.**

2. **Pre-natal developmental toxicity study (Annex X, Section 8.7.2.; test method: EU B.31./OECD TG 414) in a second species (rabbit), oral route with the registered substance;**

You may adapt the testing requested above according to the specific rules outlined in Annexes VI to X and/or according to the general rules contained in Annex XI of the REACH Regulation. In order to ensure compliance with the respective information requirement, any such adaptation will need to have a scientific justification, referring and conforming to the appropriate rules in the respective Annex, and an adequate and reliable documentation.

You are required to submit the requested information in an updated registration dossier by **8 October 2018**. You shall also update the chemical safety report, where relevant. The timeline has been set to allow for sequential testing.

The reasons of this decision are set out in Appendix 1. The procedural history is described in Appendix 2. Advice and further observations are provided in Appendix 3.

Appeal

This decision can be appealed to the Board of Appeal of ECHA within three months of its notification. An appeal, together with the grounds thereof, shall be submitted to ECHA in writing. An appeal has suspensive effect and is subject to a fee. Further details are described under http://echa.europa.eu/regulations/appeals.

Authorised[1] by Claudio Carlon, Head of Unit, Evaluation E2

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

Appendix 1: Reasons

1. In vivo mammalian alkaline comet assay (Annex X, Section 8.4., column 2)

Pursuant to Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at more than 1000 tonnes per year shall contain as a minimum the information specified in Annexes VII to X of the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.

"Mutagenicity" is an information requirement as laid down in Annex VIII, Section 8.4. of the REACH Regulation. Column 2, of Annex X, Section 8.4., provides that "If there is a positive result in any of the *in vitro* genotoxicity studies in Annexes VII or VIII, a second *in vivo* somatic cell test may be necessary, depending on the quality and relevance of all the available data."

The technical dossier contains one *in vitro* key study on the "*Induction of chromosome aberrations by styrene and vinyl acetate in cultured human lymphocytes: dependence on erythrocytes*" (Jantunen *et al.*, 1986) performed according to OECD TG 473 with the registered substance that showed positive results. According to the study there "*was a clear dose-dependent increase in chromatid breaks, gaps and total aberrations at concentrations of 0.25 mM and above.*" Furthermore, "*[t]he clastogenic effects of vinyl acetate were more pronounced in isolated lymphocytes than in whole blood up to 5 mM.*"

You also provided an *in vitro* supporting study on the "*High Content Cytotoxicity and Micronucleus Assay in Human TK6 Cells Exposed to Vinyl Acetate (CAS No.108-05-4) and Acetaldehyde (CAS No. 75-07-0)*" (ILS, 2010), performed according to OECD TG 487, that also showed positive results. According to this study the "*vinyl acetate and acetaldehyde induced a positive increase in the induction of micronucleus (MN) at levels of vinyl acetate or acetaldehyde exposure that induced <55±5% cytotoxicity based on relative survival of TK6 cells compared to unexposed controls. Vinyl acetate exposure levels of 0.25, 0.5, 1.0 and 2 mM were considered to be positive for MN induction.*" The results of this study are consistent with the findings of the key study as indicated above.

The positive results indicate that the substance is inducing chromosomal aberrations *in vitro* under the conditions of the tests.

The technical dossier contains two *in vivo* studies:

1. "*In vivo* micronucleus test on erythrocytes" (Maki-Paakkanen, 1987) performed according to OECD TG 474, though with a number of deviations, with the registered substance; the results of this study are considered ambiguous in the technical dossier;
2. "*In vivo* micronucleus test on spermatids" (Lahdetie, 1988), not performed according to any test guideline, with the registered substance; the results of this study are considered negative.

ECHA notes that both the *in vivo* micronucleus test on erythrocytes and the *in vivo* micronucleus test on spermatids cannot be considered adequate to follow up the positive *in vitro* chromosome aberration test and to cover the data requirement for *in vivo* chromosomal aberration, mainly because of the following reasons:

1. The *in vivo* micronucleus test on erythrocytes (Maki-Paakkanen, 1987) is neither GLP compliant nor performed according to OECD TG 474 as it has some important deviations, namely:
   i. This test follows a single administration with a sampling schedule of 30 hours after dosing, while two sampling times should have been performed;
   ii. For the number of cells analysed, 1000 polychromatic erythrocytes were scored per animal, while it should have been 2000 (TG 474, 1997) or 4000 (TG 476 2014);
   iii. The route of administration chosen to perform the test is intraperitoneal injection. This route was acceptable in the previous OECD TG 474. However, in the latest edition (2014), the intraperitoneal route "*is generally not recommended since it is not an intended route of human exposure, and should therefore only be used with specific scientific justification*." No justification was provided in the dossier on why the intraperitoneal route was used instead of another route of exposure such as dietary, drinking water, oral by gavage, etc.
   iv. About the outcome of the test: the two top doses induced a statistically significant increase in the micronucleus frequency (1000 and 2000 mg/kg bw), but these doses also induced death of treated animals (6/14 and 8/14 respectively). These doses should thus not be considered in the analysis of the results, meaning that the study should be concluded negative.
2. The *in vivo* micronucleus test on spermatids (Lahdetie, 1988) was not performed according to any test guideline (there is no test guideline for this test). This study cannot be considered adequate to follow up the positive *in vitro* chromosome aberration test because it does not investigate the genotoxicity on somatic cells. The negative result obtained in the micronucleus on spermatids does not rule out the possibility that a positive result is obtained on somatic cells at the site of contact.

As explained above, ECHA considers that both *in vivo* studies cannot be considered adequate to follow up the positive *in vitro* chromosome aberration test. Hence, ECHA concludes that the tests provided were not appropriate to follow-up a concern for chromosomal aberrations.

An appropriate somatic *in vivo* genotoxicity study to follow up the concern on chromosomal aberrations is not available for the registered substance but is necessary to meet the information requirements. Consequently there is an information gap and it is necessary to provide information for this endpoint.

According to the ECHA *Guidance on information requirements and chemical safety assessment* (version 5.0, December 2016) Chapter R.7a, section R.7.7.6.3, the *in vivo* mammalian alkaline comet assay ("comet assay", OECD TG 489) is suitable to follow up positive *in vitro* result showing chromosomal aberrations. ECHA considers this test to be most appropriate for the substance subject to the decision.

According to the test method OECD TG 489, the test shall be performed in rats.

Having considered the anticipated routes of human exposure performance of the test by the inhalation route appears appropriate.

However, it is considered that the most appropriate route to be used for the comet assay should be the oral route, for the following reasons. The substance is very reactive and *in vivo* studies show that it causes effects at the site of first contact. Information provided in the dossier under the carcinogenicity endpoint, as well as the conclusion of the EU RAR on vinyl acetate (2008), show that, in inhalation carcinogenicity studies on rats, vinyl acetate induces tumours only in nasal cavity, and not tracheal or lung tumours. It was thus first considered that nasal tissue would be the most relevant site of contact tissue for a comet assay by inhalation on vinyl acetate. However, the performance of the comet in nasal tissue following inhalation treatment is associated with technical challenges (e.g. collection of tissue, choice of adequate positive control). It is noted that this substance induced tumours in carcinogenicity studies both after administration by inhalation route and by oral route. For both routes, the induced tumours were observed in site of contact tissues, i.e. nasal cavity for inhalation route, and oral cavity, oesophagus, and stomach for oral route. In order to follow-up the concern observed *in vitro*, and to investigate *in vivo* the genotoxic hazard related to exposure to vinyl acetate (while still avoiding the technical challenges related to the inhalation comet assay on nasal cavity), administration by the oral route (by gavage) is considered the most appropriate for the performance of the comet assay for this substance. In order to limit hydrolysis of vinyl acetate prior to administration, the test material used should be freshly prepared.

According to the test method OECD TG 489, the comet assay should be performed by analysing tissues from liver as it is a primary site of xenobiotic metabolism, glandular stomach and duodenum as sites of direct contact. There are several expected or possible variables between the glandular stomach and the duodenum (different tissue structure and function, different pH conditions, variable physico-chemical properties and fate of the substance, and probable different local absorption rates of the substance and its possible breakdown product(s)). In light of these expected or possible variables, it is necessary to sample both tissues to ensure a sufficient evaluation of the potential for genotoxicity at the site of contact in the gastro-intestinal tract.

You provided comments on the draft decision. ECHA understands that you have sought to adapt the information requirement by reference to

i) an adaptation according to Annex XI 1.1.2, "Data on human health and environmental properties from experiments not carried out according to GLP or the test methods referred to in Article 13(3)", which applies if 4 conditions are met; and

ii) the preamble of Annex X "Before new tests are carried out to determine the properties listed in this Annex, all available in vitro data, in vivo data, historical human data, data from valid (Q)SARs and data from structurally related substances (read-across approach) shall be assessed first."

Your conclusion was: "*Based on the available data, the ECHA's request to conduct an alkaline comet assay in rats (via inhalation) is not appropriate given that vinyl acetate and acetaldehyde produce DNA cross-links and the OECD TG 489 guidelines acknowledge that the assay is not relevant for such substances.*

Since there is not another in vivo assay with accepted guidelines under REACH that can evaluate mutagenicity at the site of contact; therefore, an adaptation to the technical requirements should be granted."

In relation to point i) above, ECHA notes that you did not discuss or demonstrate that the 4 conditions that need to be met are fulfilled for any of the individual studies in the current case.

However, in the detailed justification included in your comments, you provided several arguments under the following headers:

a) the comet assay is limited to assess the genotoxicity of vinyl acetate given the production of DNA cross-links;
b) acceptable in vivo mutation assays relevant to vinyl acetate, not previously entered into the dossier, are available and satisfy ECHA's request for an in vivo mutation study;
c) highlights from a review of Albertini (2013) on the genotoxicity of vinyl acetate and acetaldehyde addressing the formation of DNA-DNA and DNA protein cross links;
d) information that transgenic models have been investigated.

You then concluded that ECHA's request to conduct a comet assay is not appropriate and that an adaptation to the technical requirements should be granted. ECHA's understanding of your approach is that, instead of using Annex XI 1.1.2., you have sought an adaptation according to Annex XI 1.2, Weight of evidence, to fulfil the data requirement.

In relation to the elements presented in sections a), b), c) and d) of the 'Detailed justification' in your comment:

a) ECHA acknowledges that the Comet assay as described by the OECD TG 489 is not appropriate to detect the effects of cross-linking agents.
b) You provided scientific documentation showing that: in carboxylesterase-competent cell lines or tissues, vinyl acetate undergoes rapid metabolism to acetaldehyde; in absence of this transformation to acetaldehyde, vinyl acetate did not induce detectable genotoxicity in vitro; the genotoxicity of vinyl acetate seems to be driven by the genotoxicity of acetaldehyde. However, ECHA considers that the additional data provided in your comments (and not previously available in the dossier) do not fulfil ECHA's request for an in vivo mutation study because the additional tests results provided do not address the genotoxicity of vinyl acetate in an in vivo biological system.
c) The detailed highlights from Albertini (2013) do not provide additional information on the in vivo genotoxicity of vinyl acetate;
d) ECHA acknowledges that transgenic models have been investigated and wish to inform that recent progress enabled the transgenic rodent gene mutation assay (OECD 488) to be performed on nasal mucosa. However, such test would not be suitable in the current case because it would investigate the gene mutation mechanism and not chromosomal aberration which is the relevant mechanism for vinyl acetate.

ECHA therefore considers that you did not provide elements demonstrating that cross-linking is the exclusive genotoxic mode of action of vinyl acetate or acetaldehyde. Considering that other genotoxic pathways have not been ruled out, the performance of a comet assay on vinyl acetate remains relevant. Moreover, no in vivo genotoxicity data are available on a site of contact tissue, and it cannot be excluded that genotoxic effects are induced at the site of first contact.

ECHA further considers that there is not sufficient weight of evidence from several independent sources of information leading to the assumption/conclusion that a substance has or has not a particular dangerous property and, specifically, that the registered substance exclusively induces DNA cross-links. ECHA considers your conclusion that the alkaline comet assay in rats is not relevant is not sufficiently justified. ECHA considers that your adaptation cannot be accepted, and a data gap remains.

Following the proposal for amendment made by one Member State Competent Authority, ECHA considers that it is important to take into account the cross-linking properties of the registered substance and its metabolite acetaldehyde in the experimental setup of the comet assay. Therefore, you are requested to prepare and analyse two sets of slides when performing the comet assay: one set will be submitted to the standard experimental conditions ; the other set of slides will be submitted to modified experimental conditions that enable the detection of DNA crosslinks by inducing additional DNA damage via treatment with MMS or ionising radiations (gamma or X-ray) according to a reliable protocol, e.g. as described in the references 36-39 of the TG 489[2] or Pant et al (2015)[3].

The modified protocol to detect crosslinks shall include a positive control to ensure the robustness of the test result obtained on the registered substance: an additional group of animals shall be treated with a known cross-linking substance (e.g. hexamethyl phosphoramide or cisplatine).

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision:

In vivo mammalian alkaline comet assay (test method: OECD TG 489) in rats, oral route, by gavage, on the following tissues: liver, glandular stomach and duodenum; two sets of slides shall be prepared and analysed, one set submitted to standard experimental conditions and one set submitted to modified experimental conditions that enable the detection of DNA crosslinks. The modified protocol shall include treatment by MMS (methyl methanesulfonate) or by ionising irradiation (according to e.g. references 36-39 in the TG 489, or Pant et al 2015) as well as a specific positive control group of animals. The test material used should be freshly prepared.

Notes for your consideration

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

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

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[2] References provided in OECD TG 489 (2016):
(36) Merk, O., G. Speit (1999), Detection of crosslinks with the Comet assay in relationship to genotoxicity and cytotoxicity, Environmental and Molecular Mutagenesis, Vol. 33/2, pp. 167-72;
(37) Pfuhler, S., H.U. Wolf (1996), Detection of DNA-crosslinking agents with the alkaline Comet assay, Environmental and Molecular Mutagenesis, Vol. 27/3, pp. 196-201;
(38) Wu, J.H., N.J. Jones (2012), Assessment of DNA interstrand crosslinks using the modified alkaline Comet assay, Methods in Molecular Biology, Vol. 817, pp. 165-81;
(39) Spanswick, V.J., J.M. Hartley, J.A. Hartley (2010), Measurement of DNA interstrand crosslinking in individual cells using the Single Cell Gel Electrophoresis (Comet) assay, Methods in Molecular Biology, Vol. 613, pp. 267-282.
[3] Pant K, Roden N, Zhang C, Bruce C, Wood C, and Pendino K (2015) Modified In Vivo Comet Assay Detects the Genotoxic Potential of14-Hydroxycodeinone, an a,b-Unsaturated Ketone in Oxycodone. Environmental and Molecular Mutagenesis 56, 777-787.

2. Pre-natal developmental toxicity study (Annex X, Section 8.7.2.) in a second species

Pursuant to Articles 10(a) and 12(1) of the REACH Regulation, a technical dossier registered at more than 1000 tonnes per year shall contain as a minimum the information specified in Annexes VII to X of the REACH Regulation. The information to be generated for the dossier must fulfil the criteria in Article 13(4) of the same regulation.

Pre-natal developmental toxicity studies (test method EU B.31./OECD TG 414) on two species are part of the standard information requirements for a substance registered for 1000 tonnes or more per year (Annex IX, Section 8.7.2., column 1, Annex X, Section 8.7.2., column 1, and sentence 2 of introductory paragraph 2 of Annex X of the REACH Regulation).

The technical dossier contains information on a pre-natal developmental toxicity study in rats by the oral and inhalation route using the registered substance as test material. However, there is no information provided for a pre-natal developmental toxicity study in a second species. Consequently there is an information gap and it is necessary to provide information for this endpoint.

You provided comments on the draft decision, in which you particularly argue an adaptation according to Weight of Evidence (Annex XI, 1.2) to fulfil the information requirement of Annex X, 8.7.2.

Since you mention in these comments "ECHA's statement" that pre-natal developmental toxicity studies on two species are part of the standard information requirements for a substance registered for 1000 tons or more per year in this context, please note that the Board of Appeal of ECHA in case A-004-2012 clarified that ECHA correctly interpreted the REACH Regulation in the way that Annex X, Section 8.7.2. of the REACH Regulation stipulates an additional standard information requirement for a pre-natal developmental toxicity study in a second species. ECHA is aware of the applications for review pending before the Court of Justice of the European Union, but it considers that these have no immediate effect and no conclusions can be drawn before a ruling of the Court on the matter.

ECHA has assessed the weight of evidence according to Annex XI, 1.2. Firstly, ECHA has assessed the individual sources of information (with reference to the labelling in your comment, a.) to e.)), and subsequently ECHA has assessed the weight of evidence.

a) You refer to sub-chronic studies in rats and mice. ECHA considers that these studies do not address key parameters of the OECD 414 study (e.g. treatment of a sufficient number of pregnant animals, foetal examinations), and do not address a key parameter of the information requirement, i.e. results in a second, non-rodent species.
b) You refer to two PNDT studies in rats. ECHA considers that these studies do not address a key parameter of the information requirement, i.e. results in a second, non-rodent species.
c) You refer to a 2-generation study in rats. ECHA considers that these studies do not address key parameters of the OECD 414 study (e.g. foetal examinations), and do not address a key parameter of the information requirement, i.e. results in a second, non-rodent species.

d) You refer to an OECD 422 study in rats on the read-across substance, acetaldehyde. ECHA notes this study is not available in the dossier, and ECHA can only provide preliminary comments. The read-across justification is not provided, and so ECHA cannot evaluate the relevance of this study on a read-across chemical. ECHA considers that these studies do not address key parameters of the OECD 414 study (e.g. treatment of a sufficient number of pregnant animals, foetal examinations), and do not address a key parameter of the information requirement, i.e. results in a second, non-rodent species.
e) You refer to a teratogenicity study with acetaldehyde in rats. ECHA notes this study is not available in the dossier, and ECHA can only provide preliminary comments. The read-across justification is not provided, and so ECHA cannot evaluate the relevance of this study on a read-across chemical. ECHA considers that this study does not address a key parameter of the information requirement, i.e. results in a second, non-rodent species.

You state that "The above summary of available data, taken together in a weight of evidence assessment indicates that vinyl acetate and/or acetaldehyde (its reactive metabolite) is of negligible or none concern for developmental toxicity." However, ECHA considers that this is merely an assertion, and is not a reasoned justification of why there is sufficient weight of evidence for this information requirement. ECHA considers that you have not provided justification of why there is sufficient weight of evidence for this information requirement, and ECHA considers that you have failed to provide adequate and reliable documentation of their weight of evidence adaptation. Further ECHA considers that there is not sufficient weight of evidence from several independent sources of information leading to the assumption/ conclusion that a substance has or has not a particular dangerous property for this information requirement. ECHA considers that your adaptation cannot be accepted, and a data gap remains.

The test in the first species was carried out by using a rodent species (rats). According to the test method EU B.31./OECD 414, the rabbit is the preferred non-rodent species. On the basis of this default assumption, ECHA considers that the test should be performed with rabbits as a second species.

ECHA considers that the oral route is the most appropriate route of administration for substances except gases to focus on the detection of hazardous properties on reproduction as indicated in ECHA *Guidance on information requirements and chemical safety assessment* (version 5.0, December 2016) R.7a, chapter R.7.6.2.3.2. Since the substance to be tested is a liquid, ECHA concludes that testing should be performed by the oral route.

Therefore, pursuant to Article 41(1) and (3) of the REACH Regulation, you are requested to submit the following information derived with the registered substance subject to the present decision: Pre-natal developmental toxicity study (test method: EU B.31./OECD TG 414) in a second species (rabbit) by the oral route.

Appendix 2: Procedural history

For the purpose of the decision-making, this decision does not take into account any updates of your registration after the date when the draft decision was notified to you under Article 50(1) of the REACH Regulation.

The compliance check was initiated on 24 March 2016.

The decision making followed the procedure of Articles 50 and 51 of the REACH Regulation, as described below:

ECHA notified you of the draft decision and invited you to provide comments.

ECHA took into account your comments and did not amend the request(s).

ECHA notified the draft decision to the competent authorities of the Member States for proposals for amendment.

ECHA received proposal(s) for amendment and modified the draft decision.

ECHA invited you to comment on the proposed amendment(s).

ECHA referred the draft decision to the Member State Committee.

Your comments on the proposed amendment(s) were taken into account by the Member State Committee.

The Member State Committee reached a unanimous agreement on the draft decision during its MSC-51 meeting and ECHA took the decision according to Article 51(6) of the REACH Regulation.

Appendix 3: Further information, observations and technical guidance

1. The substance subject to the present decision is provisionally listed in the Community rolling action plan (CoRAP) for start of substance evaluation in 2018.

2. This compliance check decision does not prevent ECHA from initiating further compliance checks on the present registration at a later stage.

3. Failure to comply with the request(s) in this decision, or to fulfil otherwise the information requirement(s) with a valid and documented adaptation, will result in a notification to the enforcement authorities of your Member State.

4. In relation to the information required by the present decision, the sample of the substance used for the new test(s) must be suitable for use by all the joint registrants. Hence, the sample should have a composition that is suitable to fulfil the information requirement for the range of substance compositions manufactured or imported by the joint registrants. It is the responsibility of all joint registrants who manufacture or import the same substance to agree on the appropriate composition of the test material and to document the necessary information on their substance composition. In addition, it is important to ensure that the particular sample of the substance tested in the new test(s) 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 or imported by each registrant. If the registration of the substance by any registrant covers different grades, the sample used for the new test(s) must be suitable to assess these grades. Finally there must be adequate information on substance identity for the sample tested and the grade(s) registered to enable the relevance of the test(s) to be assessed.