~~Confidential~~

Helsinki, 10 February 2022

Addressees
Registrant(s) of JS-2-Vinylpyridine as listed in the last Appendix of this decision

Date of submission of the dossier subject to this decision
03/09/2010

Registered substance subject to this decision ("the Substance")
Substance name: 2-vinylpyridine
EC number: 202-879-8
CAS number: 100-69-6

Decision number: Please refer to the REACH-IT message which delivered this communication (in format CCH-D-XXXXXXXXXX-XX-XX/F)

DECISION ON A COMPLIANCE CHECK

Under Article 41 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed below, by the deadline of ***15 November 2024***.

Requested information must be generated using the Substance unless otherwise specified.

A. Information required from all the Registrants subject to Annex VIII of REACH

1. In vivo mammalian alkaline comet assay combined with in vivo mammalian erythrocyte micronucleus test also requested below (triggered by Annex VIII, Section 8.4., column 2; test method: OECD TG 489)

2. Justification for an adaptation of a Screening for reproductive/developmental toxicity based on the results of the Extended one-generation reproductive toxicity study requested below (Annex VIII, Section 8.7.1.)

B. Information required from all the Registrants subject to Annex IX of REACH

1. In vivo mammalian alkaline comet assay (triggered by Annex VIII, Section 8.4., column 2; test method: OECD TG 489) combined with in vivo mammalian erythrocyte micronucleus test (test method: OECD TG 474) in rats, oral route. For the comet assay the following tissues shall be analysed: liver, glandular stomach and duodenum.

2. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.; test method: OECD TG 414) by oral route, in one species (rat or rabbit)

3. Long-term toxicity testing on fish (Annex IX, Section 9.1.6.; test method: OECD TG 210)

C. Information required from all the Registrants subject to Annex X of REACH

1. Pre-natal developmental toxicity study (Annex X, Section 8.7.2.; test method: OECD TG 414) by oral route, in a second species (rat or rabbit)

2. Extended one-generation reproductive toxicity study (Annex X, Section 8.7.3.; test method: OECD TG 443) by oral route, in rats, specified as follows:

   - Ten weeks premating exposure duration for the parental (P0) generation;
   - Dose level setting shall aim to induce systemic toxicity at the highest dose level;
   - Cohort 1A (Reproductive toxicity);
   - Cohort 1B (Reproductive toxicity) without extension to mate the Cohort 1B animals to produce the F2 generation

   You must report the study performed according to the above specifications. Any expansion of the study must be scientifically justified.

Reasons for the request(s) are explained in the following appendices:

- Appendix entitled "Reasons common to several requests";
- Appendices entitled "Reasons to request information required under Annexes VIII to X of REACH", respectively.

Information required depends on your tonnage band

You must provide the information listed above for all REACH Annexes applicable to you, and in accordance with Articles 10(a) and 12(1) of REACH:

- the information specified in Annexes VII and VIII to REACH, for registration at 10-100 tpa;
- the information specified in Annexes VII, VIII and IX to REACH, for registration at 100-1000 tpa;
- the information specified in Annexes VII to X to REACH, for registration at more than 1000 tpa.

You are only required to share the costs of information that you must submit to fulfil your information requirements.

For certain endpoints, ECHA requests the same study from registrants at different tonnages. In such cases, only the reasoning why the information is required at lower tonnages is provided in the corresponding Appendices. For the tonnage where the study is a standard information requirement, the full reasoning for the request including study design is given. Only one study is to be conducted; the registrants concerned must make every effort to reach an agreement as to who is to carry out the study on behalf of the other registrants under Article 53 of REACH.

How to comply with your information requirements

To comply with your information requirements you must submit the information requested by this decision in an updated registration dossier by the deadline indicated above. You must also update the chemical safety report, where relevant, including any changes to classification and labelling, based on the newly generated information.

You must follow the general testing and reporting requirements provided under the Appendix entitled "Requirements to fulfil when conducting and reporting new tests for REACH purposes". For references used in this decision, please consult the Appendix entitled "List of references".

Appeal

This decision, when adopted under Article 51 of REACH, may be appealed to the Board of Appeal of ECHA within three months of its notification to you. Please refer to http://echa.europa.eu/regulations/appeals for further information.

Failure to comply

If you do not comply with the information required by this decision by the deadline indicated above, ECHA will notify the enforcement authorities of your Member State.

Authorised[1] under the authority of Mike Rasenberg, Director of Hazard Assessment

[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 on Reasons common to several requests

1. Assessment of the (Q)SAR adaptation under Annex XI, Section 1.3.

You seek to adapt the following standard information requirements by applying (a) (Q)SAR approach(es) in accordance with Annex XI, Section 1.3:

- In vivo mammalian erythrocyte micronucleus test (Annex IX, Section 8.4., column 2)
- Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.)
- Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.)
- Pre-natal developmental toxicity study in a second species (Annex X, Section 8.7.2.)
- Extended one-generation reproductive toxicity study (Annex X, Section 8.7.3.)

ECHA has considered the scientific and regulatory validity of your (Q)SAR adaptation(s) in general before assessing the specific standard information requirements in the following appendices.

Under Annex XI, Section 1.3., the following conditions must be fulfilled whenever a (Q)SAR approach is used:

1. the prediction needs to be derived from a scientifically valid model,
2. the substance must fall within the applicability domain of the model,
3. results need to be adequate for the purpose of risk assessment or classification and labelling, and
4. adequate and reliable documentation of the method must be provided.

With regard to these conditions, we have identified the following issue(s):

1. Modelled endpoint not well defined

Under ECHA Guidance R.6.1.3., a (Q)SAR model must fulfil the principles described in the OECD Guidance document on the validation of (Q)SAR models (ENV/JM/MONO(2007)2) to be considered scientifically valid. The first OECD principle requires the endpoint of a (Q)SAR model to be well defined. ECHA Guidance R.6.5.1.2 specifies that for a well-defined endpoint:

- the training set must be obtained from experimental data generated with homogeneous experimental protocols, and
- the effect modelled being predicted by the (Q)SAR must be the same as the effect measured by a defined test protocol relevant to the information requirement, which in this case includes fertility, reproductive performance and effects on offspring as in OECD TG 421/422/443 and developmental effects as in OECD TG414 or induction of micronuclei OECD TG 474.

You specify that the effects that are modelled are in vivo induction of micronuclei for genetic toxicity, the "*reproductive toxicity potential*" for reprotoxicity and "*developmental toxicity*".

You have provided Cat-SAR and MC4PC version 2.1 (Q)SAR models which are based on data from different datasets, e.g. chemicals from the U.S. National Toxicology Program tested for their ability to induce micronuclei in mice or the U.S. FDA ReproTox set and the legacy reprotox set.

We have evaluated the information and identified the following issue:

For genotoxicity and developmental toxicity you have not provided information establishing that the datasets the models are based on homogenous protocols and it cannot be excluded that they were obtained from heterogeneous protocols. The dossier data does not specify if same or different species and experimental protocols were used. For reprotoxicity it is clearly stated for example that the "modern reproductive toxicity" set consists of modules with

different species for reproductive toxicity in adult males, sperm toxicity, reproductive toxicity in females.

Therefore, ECHA can not conclude whether the training set is obtained from experimental data generated with homogeneous experimental protocols.

Furthermore, none of the three endpoints predicted by the (Q)SARs are demonstrated to be the same as the endpoints measured by the relevant test protocols. This is because the predictions are qualitative for all three endpoints predicted. In addition, for reproductive and developmental toxicity it is not known what the conclusion of "(non) reproductive/developmental toxicity" is based on. The datasets consider data from different species and experimental protocols to make the overall conclusion. The specific effects of the substance modelled for any of the predicted endpoints are not known, neither is a NOAEL (relevant for Reproductive and developmental effects) or any underlying data (all endpoints). Therefore the endpoint of the model is not well defined and you have not established that the use of this model is a scientifically valid approach to meet these information requirements.

2. *Inadequate documentation of the model (QMRF)*

Under Appendix C of the OECD Guidance document on the validation of (Q)SAR models (ENV/JM/MONO(2007)2) and ECHA Guidance R.6.1.6.3., adequate and reliable documentation must include a (Q)SAR Model Reporting Format document (QMRF) which reports, among others, the following information:

- the predicted endpoint, including information on experimental protocol and data quality for the data used to develop the model;

For all three predicted endpoints, detailed information on all three predicted endpoints, the experimental protocol and data quality for the data used to develop the models are missing. Information on the full datasets is not available.

In absence of such information, ECHA cannot establish that the model can be used to meet these information requirements.

3. *Lack of or inadequate documentation of the prediction (QPRF)*

ECHA Guidance R.6.1.6.3 states that the information specified in or equivalent to the (Q)SAR Prediction Reporting Format document (QPRF) must be provided to have adequate and reliable documentation of the applied method. For a QPRF this includes, among others:

- the model prediction(s), including the endpoint,
- the relationship between the modelled substance and the defined applicability domain,
- the identities of close analogues, including considerations on how predicted and experimental data for analogues support the prediction.

As already addressed above, the prediction is qualitative and it is not demonstrated that the datasets, the models are based on, are homogenous. Therefore, it is not known from the documentation how the model predictions and the overall conclusions were derived for all three predicted endpoints.

Detailed information on how fragments were considered to derive domain definitions is not provided. For Cat-SAR models, the applicability domain is determined on a one-by-one basis. Fragments are generated from the target molecule and compared with all possible fragments of the substances in the training set. A list of fragments meeting the model's criteria is produced and the proportion of active to inactive compounds from each fragment identified in the compound is the metric of activity. Therefore no specific applicability domain can be

defined and thus neither the relationship between the modelled substance and the defined applicability domain for all three predicted endpoints .

The information on close analogues does not include considerations on how predicted and experimental data for analogues may support the prediction for all three predicted endpoints. In particular the analogues for some reprotoxicity models are aggregated on higher levels than individual predictions. For developmental toxicity there is no explanation how the analogues, differing in functional groups from the Substance, support the prediction.

For reprotoxicity, the documentation solely refers to "CASE units" and a multitude of endpoints, mixing developmental and reproductive toxicity.

In absence of such information, ECHA cannot establish that the prediction can be used to meet these information requirements.

Therefore, your adaptations are rejected.

Appendix A: Reasons to request information required under Annex VIII of REACH

1. **In vivo mammalian alkaline comet assay (triggered by Annex VIII, Section 8.4., column 2; test method: OECD TG 489) combined with in vivo mammalian erythrocyte micronucleus test (test method: OECD TG 474) in rats, oral route. For the comet assay the following tissues shall be analysed: liver, oral: glandular stomach and duodenum.**

Under Annex VIII, Section 8.4, column 2 of REACH, the performance of an appropriate *in vivo* somatic cell genotoxicity study must be considered if there is a positive result in any of the *in vitro* genotoxicity studies in Annex VII or VIII.

The ECHA guidance R.7a states that following a positive result in an *in vitro* test, "*adequately conducted somatic cell in vivo testing is required to ascertain if this potential can be expressed in vivo. In cases where it can be sufficiently deduced that a positive in vitro finding is not relevant for in vivo situations (e.g. due to the effect of the test substances on pH or cell viability, in vitro-specific metabolism: see also Section R.7.7.4.1), or where a clear threshold mechanism coming into play only at high concentrations that will not be reached in vivo has been identified (e.g. damage to non-DNA targets at high concentrations), in vivo testing will not be necessary.*"

Your dossier contains positive results for the *in vitro* gene mutation study in bacteria and *in vitro* cytogenicity tests which raise the concerns for gene mutation and chromosomal aberration. Further, there is no indication that a positive *in vitro* finding is not relevant for in vivo situations or that a clear threshold mechanism comes into play only at high concentrations that will not be reached in vivo has been identified. Therefore, the trigger to provide in vivo genotoxicity study is met.

The information provided to fulfil the in vivo information requirement and the study design are addressed below under Section B.1. (on the Annex IX requirement).

In the comments to the draft decision, you agree to perform the requested study.

2. **Justification for an adaptation of a Screening for reproductive / developmental toxicity based on the results of the Extended one-generation reproductive toxicity study**

Screening for reproductive/developmental toxicity is a standard information requirement under Annex VIII to REACH. This information may take the form of a study record or a valid adaptation in accordance with either a specific adaptation rule under Column 2 of Annex VIII or a general adaptation rule under Annex XI.

You have provided a weight of evidence adaptation.

In support of your adaptation, you have provided the following source of information:

(i) 2010, a QSAR prediction with the MC4PC software and two sets of carefully designed expert modules. (legacy repro set (rat, mouse, rabbit and humans) and modern repro set (tests for Reproductive toxicity in adult males, sperm toxicity and reproductive toxicity in females);

(ii) 1984, a subchronic repeated dose toxicity study, similar to OECD TG 408.

Based on the presented sources of information, you argue that the available data gives sufficient information to conclude on the reproductive toxcity.

Annex XI, Section 1.2 states that there may be sufficient weight of evidence weight of evidence from several independent sources of information leading to assumption/conclusion

that a substance has or has not a particular dangerous (hazardous) property, while information from a single source alone is insufficient to support this notion.

According to ECHA Guidance R.4, a weight of evidence adaptation involves an assessment of the relative values/weights of the different sources of information submitted. The weight given is based on the reliability of the data, consistency of results/data, nature and severity of effects, and relevance and coverage of the information for the given regulatory information requirement. Subsequently, relevance, reliability, coverage, consistency and results of these sources of information must be balanced in order to decide whether they together provide sufficient weight to conclude that the Substance has or has not the (dangerous) property investigated by the required study.

Annex XI, section 1.2 requires that adequate and reliable documentation is provided to describe your weight of evidence adaptation.

You have not submitted any explanation why the sources of information provide sufficient weight of evidence leading to the conclusion/assumption that the Substance has or has not a particular dangerous property investigated by the required study.

Irrespective of the above mentioned deficiencies on the documentation, which in itself could lead to the rejection of the adaptation, ECHA has assessed the provided sources of information.

Relevant information that can be used to support weight of evidence adaptation for information requirement of Section 8.7.1 at Annex VIII includes similar information that is produced by the OECD TG 421 or 422. At general level, it included information on 1) sexual function and fertility, 2) toxicity to offspring and examination of offspring parameter and 3) systemic toxicity.

1) Sexual function and fertility

Sexual function and fertility on both sexes must cover information on mating, fertility, gestation (length), maintenance of pregnancy (abortions, total resorptions), parturition, lactation, organ weights and histopathology of reproductive organs and tissues, oestrous cyclicity, sperm count, sperm analysis, hormone levels, litter sizes, nursing performance and other potential aspects of sexual function and fertility.

The repeated dose toxicity study (ii) provide relevant information on integrity of reproductive organs on both sexes. The study (i) provides information on structural alerts for reproductive toxicity and sperm toxicity.

The sources of information (i) and (ii) do not provide qualitative (ii) and quantitative (i) information and (ii) information on functional fertility on males and females. The repeated dose toxicity study (ii) informs only about reproductive organs without mating of animals.

Therefore there is only partially information on sexual function and fertility.

However, the following deficiencies affect the reliability of these sources of information.

Functional fertility and histopathology of reproductive organs and tissues must be investigated in parental P0 animals as indicated in OECD TG 441/422 after at least 4 weeks for males and 9 weeks for females premating exposure duration. The sources of information (i) and (ii) do not cover the full duration as defined in OECD TG 421/422.

In the absence of absence of reliable information sexual function and fertility with sufficient premating exposure duration for both parental P0 animals, no conclusion can be drawn on sexual function and fertility as required by the information requirement.

In addition, as explained in the Appendix on reasons common to several requests, the reported Q(SAR) approach is not reliable.

2) Toxicity to offspring

Toxicity to offspring must cover information on deaths before, during or after birth, growth, external malformations, clinical signs, sexual maturity, oestrous cyclicity, histopathology of reproductive organs in adulthood and other potential aspects of toxicity to offspring.

The source of information (ii) investigates adult animals without producing offspring and therefore are lacking information on offspring. The source of information (i) does not provide relevant information to deaths before, during or after birth, on growth, external malformations, clinical signs, sexual maturity, oestrous cyclicity, histopathology of reproductive organs in adulthood and other potential aspects of toxicity to offspring.

Therefore, no reliable information on toxicity to offspring up to the adulthood is available and in the absence of this no conclusion can be drawn on toxicity to offspring as required by the information requirement.

Therefore, only source of information (i) provide relevant information and only partially. It is, however, not reliable as mentioned above.

3) Systemic toxicity

Information on general organ toxicity, haematology and clinical chemistry is available from the provided study (ii) for parental animals but not from source (i).

Therefore, only source of information (ii) provides relevant information on systemic toxicity of adult animals.

Taken together, the relevant sources of information as indicated above, provide information on systemic toxicity but only partial information on sexual function and fertility, toxicity to offspring, while the information provided is not reliable.

Therefore, it is not possible to conclude, based on any source of information alone or considered together, whether your Substance has or has not the particular dangerous properties foreseen to be investigated in an OECD TG 421/422 study. Therefore, your adaptation is rejected, and the information requirement is not fulfilled.

The present decision requests the registrants concerned to generate and submit an extended one-generation reproductive toxicity study (EOGRTS) (see Section D.2). Once an EOGRTS is available, according to Column 2 of Annex VIII, Section 8.7.1. and to prevent unnecessary animal testing, a screening for reproductive/developmental toxicity does not therefore need to be conducted. While you still have to comply with the information requirement in Annex VIII, Section 8.7.1., you are requested to submit a justification for the adaptation based on Column 2 of that provision.

In the comments to the draft decision, you indicate your intention to use the Extended One-Generation Reproductive Toxicity Study (Annex IX, Section 8.7.3; OECD TG 443), requested in the current draft decision, to adapt this information requirement.

ECHA points out that when the Extended One-Generation Reproductive Toxicity Study is available, you may adapt this information requirement according to Annex VIII, Section 8.7.1, Column 2, first paragraph, fourth indent of REACH ("*this study does not need to be conducted if: [..] an* Extended One-Generation Reproductive Toxicity Study *(Annex IX, Section 8.7.3) [..] is available*"). However, at this point in time, the study is still to be conducted.

Based on the above, the information requirement is not fulfilled.

Appendix B: Reasons to request information required under Annex IX of REACH

1. **In vivo mammalian alkaline comet assay (triggered by Annex VIII, Section 8.4., column 2; test method: OECD TG 489) combined with in vivo mammalian erythrocyte micronucleus test (test method: OECD TG 474) in rats, oral route. For the comet assay the following tissues shall be analysed: liver, oral: glandular stomach and duodenum.**

Under Annex IX, Section 8.4, column 2 of REACH, the information requirement for an appropriate *in vivo* somatic cell genotoxicity study is triggered if 1) there is a positive result in any of the *in vitro* genotoxicity studies in Annex VII or VIII and 2) there are no appropriate results already available from an *in vivo* somatic cell genotoxicity study.

In relation to the first condition, your dossier contains positive results for the *in vitro* gene mutation study in bacteria and *in vitro* cytogenicity tests which raise the concerns for gene mutation and chromosomal aberration.

In relation to the second condition, your dossier contains the following *in vivo* study

i. 1992, non-guideline study, *"A study of tobacco carcinogenesis XLVII. Bioassays of vinylpyridines for genotoxicity and for tumorigenicity in mice"* with the Substance, a lung tumour induction assay.

To be considered adequate, the study has to meet the requirements of OECD TG 474/489, and the key parameters of this test guideline include:

a) The study must include a minimum of three doses/groups of treated animals as well as a negative control group and a positive control group.
b) The highest dose studied must be the maximum tolerated dose (MTD), i.e. the highest dose that is tolerated without evidence of toxicity (e.g. body weight depression or hematopoietic system cytotoxicity, but not death or evidence of pain, suffering or distress necessitating humane euthanasia).
c) The proportion of immature among total (immature + mature) erythrocytes must be determined for each animal (by counting a total of at least 500 erythrocytes for bone marrow and 2000 erythrocytes for peripheral blood).
d) Where increases in DNA migration are observed, an examination of one or more indicators of cytotoxicity (e.g. inflammation, cell infiltration, apoptotic or necrotic changes) must be performed, as target tissue toxicity may result in increases in DNA migration.
e) At least 4000 immature erythrocytes per animal must be scored for the incidence of micronucleated immature erythrocytes.
f) At least 150 cells must be analysed for each sample (per tissue, per animal).
g) The proportion of immature erythrocytes among total erythrocytes and the mean number of micronucleated immature erythrocytes must be reported for each group of animals.
h) Data on the % tail DNA (or other measures, if chosen) and mean values per group should be reported for the treated and control groups.
i) It is not appropriate to perform this test if there is evidence that the test substances, or a relevant metabolite, will not reach the target tissue.

ECHA acknowledges that you provided an *in vivo* non guideline study (i) performed with the Substance in order to follow up the concern for gene mutation and chromosomal aberration raised by the *in vitro* results. However, the above mentioned key parameter(s) are not met, because the reported data for the study do not include:

a) the appropriate number of doses
b) a maximum studied dose that is a MTD or induces toxicity
c) the analysis of the adequate number of cells
d) a negative control with a response inside the historical control range of the laboratory
e) a positive control group (or scoring control) that produced a statistically significant increase in the induced response compared with the concurrent negative control

f) data on the proportion of immature erythrocytes among total erythrocytes and the mean number of micronucleated immature erythrocytes for each group of animals
g) data on the mitotic index and the mean number of cells with aberrations per group for each group of animals
h) data on the mutation frequency for each tissue and for the treated and control groups
i) data on the % tail DNA (or other measures, if chosen) and mean values per group for the treated and control groups.

In addition, the information requirement is for informing on a concern for cytogenicity or gene mutation. However, the provided study (i) does not inform on either of these, instead this non-validated non-guideline study informs on tumour formation. Tumour formation does not inform on germ cell mutagenicity. The information provided does not cover key parameter(s) required by OECD TG 474 and 489. Therefore, the study does not fulfil the information requirement.

Therefore, the conditions set out in Annex IX, Section 8.4, column 2 are met and the information requirement for an appropriate *in vivo* somatic cell genotoxicity study is triggered.

Therefore, the provided *in vivo* test is not appropriate.

Beside the study rejected above, your dossier contains the following waiver and QSAR study:

i. A data waiver: "2-Vinylpyridine is a corrosive substance and is classified as such. According to the Introduction of Annex VIII in Regulation (EC) No. 1907/2006, "in vivo testing with corrosive substances at concentration/dose levels causing corrosivity shall be avoided." The high dose of an in vivo micronucleus study in mice, or a comet assay in mice, or other genotoxicity studies, requires the administration of doses which demonstrate toxicity to the target organ. Therefore, the study should not be conducted as pain and suffering is expected to occur in nearly all animals. In lieu of an in vivo study, a prediction from computer modelling of micronucleus studies in mice using a validated SAR model is presented.*";*
ii. 2010, a QSAR prediction for Genotoxicity in vivo (Micronucleus);

We have assessed this information and identified the following issue(s):

1) data waiver rejected

According to paragraph 3 of the preamble of Annex VIII, testing at doses causing corrosivity must be avoided. Appropriate OECD TGs for *in vivo* tests provide rules accommodating irritative and corrosive properties by e.g. adjusting the volume of vehicle.

You claim that an in vivo study requires doses demonstrating toxicity in the target organ and this would be at a dose causing corrosivity. An existing study (1997, similar to OECD TG 407) with the Substance shows effects of systemic toxicity at doses with test substance, tested up to 200 mg/kg bw/d, without mortality or excessive suffering of test animals.

Paragraph 3 of the Preamble of Annex VIII is not a legal basis for adaptation, but sets a consideration to address when carrying out testing. Furthermore, you have not taken into account that there are ways to test corrosive substances provided by appropriate OECD TGs for *in vivo* studies, such as modulating the vehicle volume. In addition, the pH can be adjusted with buffers towards more physiological values. In any case, you have not substantiated your claim that the dose that would result in toxicity in the target organ is actually corrosive. The existing study with the Substance (1997, similar to TG 407), shows the contrary. Therefore, you have not demonstrated that an *in vivo* test cannot be performed.

Therefore, your waiver is rejected and the information requirement is not fulfilled.

2) Q(SAR) adaptation rejected

As explained in the Appendix on reasons common to several requests, the reported Q(SAR) approach adaptation does not fulfil the criteria in Annex XI, Section 1.3 and is rejected. Therefore, the study (ii.) cannot be used to fulfil the information requirement.

ECHA considers that an appropriate *in vivo* follow up mutagenicity study is necessary to address the concerns identified *in vitro*.

The positive *in vitro* results available in the dossier indicate a concern for both chromosomal aberration and gene mutation. According to the ECHA Guidance R.7a, Section R.7.7.6.3, the *in vivo* mammalian alkaline comet assay ("comet assay", OECD TG 489) is a genotoxicity indicator test that is suitable to follow up the positive *in vitro* result for both chromosomal aberration and gene mutation. However, the *in vivo* mammalian erythrocyte micronucleus test ("MN test", OECD TG 474) is a mutagenicity test that provides evidence of *in vivo* chromosomal mutagenicity, as the study detects both structural and numerical chromosomal aberrations. As also indicated in the ECHA Guidance, it is possible to combine the comet assay and the MN test into a single study. The combined study can help reduce the number of tests performed and the number of animals used while addressing both chromosomal aberration and gene mutation. Therefore, the comet assay combined with the MN test is the most appropriate study for the Substance.

According to the test method OECD TG 489, the test must be performed in rats. Therefore, the combined test (OECD TG 489 and OECD TG 474) must be performed in rats. Having considered the anticipated routes of human exposure and the need for adequate exposure of the target tissue(s) performance of the test by the oral route is appropriate.

In line with the test method OECD TG 489, the test must be performed by analysing tissues from liver as primary site of xenobiotic metabolism, glandular stomach and duodenum as sites of 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 analyse both tissues to ensure a sufficient evaluation of the potential for genotoxicity at the site of contact in the gastro-intestinal tract.

i. Germ cells

A subsequent germ cell genotoxicity study (TGR/OECD TG 488, or CA on spermatogonia/OECD TG 483, depending on the concern raised by the substance) may still be required under Annex IX/X of REACH, in case 1) an *in vivo* genotoxicity test on somatic cell is positive, and 2) no clear conclusion can be made on germ cell mutagenicity.

You may consider collecting the male gonadal cells collected from the seminiferous tubules in addition to the other aforementioned tissues in the comet assay, as it would optimise the use of animals. You can prepare the slides for male gonadal cells and store them for up to 2 months, at room temperature, in dry conditions and protected from light. Following the generation and analysis of data on somatic cells in the comet assay, in accordance to Annex IX, Section 8.4., column 2, you should consider analysing the slides prepared with gonadal cells. 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.

In the comments to the draft decision, you agree to perform the requested study.

2. Pre-natal developmental toxicity study in one species

A Pre-natal developmental toxicity (PNDT) study (OECD TG 414) in one species is a standard information requirement under Annex IX to REACH.

You have provided a QSAR adaptation using the following information:

(i) 2010, Qualitative SAR prediction, Cat-SAR Human Developmental Toxicity-2-Vinylpyridine

We have assessed this information and identified the following issue:

A. Assessment of your (Q)SAR adaptation

As explained in the Appendix on reasons common to several requests, your adaptation is rejected.

Based on the above, the information you provided does not fulfil the information requirement.

A PNDT study according to the test method OECD TG 414 must be performed in rat or rabbit as preferred species with oral[2] administration of the Substance.

3. Long-term toxicity testing on fish

Long-term toxicity testing on fish is an information requirement under Annex IX to REACH (Section 9.1.6.).

You have provided the following information:

- a justification to omit the study which you consider to be based on Annex IX, Section 9.1.6, Column 2. In support of your adaptation, you provided the following justification: *"According to Regulation (EC) No. 1907/2006, Annex IX, Column 2, 9.1.6, long term toxicity testing shall be proposed if the chemical safety assessment according to Annex 1 indicates the need to investigate further the effects on aquatic organisms. Additional testing in vertebrates is not indicated based on the moderate acute toxicity of 2VP. 2VP is classified as Chronic Category 2 and its release into the environment will be minimized."*

We have assessed this information and identified the following issue:

Annex IX, Section 9.1.6, Column 2 does not allow omitting the need to submit information on long-term toxicity to fish under Column 1. It must be understood as a trigger for providing further information on fish if the chemical safety assessment according to Annex I indicates the need (Decision of the Board of Appeal in case A-011-2018).

Your adaptation is therefore rejected.

Study design

To fulfil the information requirement for the Substance, the Fish, Early-life Stage Toxicity Test (test method OECD TG 210) is the most appropriate (ECHA Guidance R.7.8.2.).

[2] ECHA Guidance R.7a, Section R.7.6.2.3.2.

Appendix C: Reasons to request information required under Annex X of REACH

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

Pre-natal developmental toxicity (PNDT) studies (OECD TG 414) in two species is a standard information requirement under Annex X to REACH.

You have provided a QSAR adaptation using the following information:

(i) 2010, Qualitative SAR prediction, Cat-SAR Human Developmental Toxicity-2-Vinylpyridine

We have assessed this information and identified the following issue(s):

A. Assessment of your (Q)SAR adaptation

As explained in the Appendix on reasons common to several requests, your adaptation is rejected.

Based on the above, the information you provided does not fulfil the information requirement.

Information on study design

A PNDT study according to the OECD TG 414 study should be performed in the rabbit or rat as the preferred second species, depending on the species tested in the first PNDT study (request B.2 in this decision).

The study shall be performed with oral[3] administration of the Substance.

2. Extended one-generation reproductive toxicity study (Annex X, Section 8.7.3.)

The basic test design of an Extended one-generation reproductive toxicity (EOGRT) study (OECD TG 443) is a standard information requirement under Annex X to REACH. Furthermore Column 2 of Section 8.7.3. defines when the study design needs to be expanded.

You have provided an adaptation under Section 8.7.3, Column 1, Annex IX arguing that there is no indication of adverse effect: *According to Regulation (EC) No.1907/2006, Annex IX, Columns 1- 2, a two-generation reproductive toxicity study is required if the 28-day or 90-day study indicates adverse effects on reproductive organs or tissues. The weight of evidence of results of subchronic studies and computer model predictions of similarly-structured substances is that 2VP is not a reproductive toxicant. The Column 1 criteria is met and thus the requirement for a two-generation reproductive toxicity study is adapted.",*

In support of your adaptation, you have provided the following sources of information:

(i) 2010, a QSAR prediction with the MC4PC software and two sets of carefully designed expert modules. (legacy repro set (rat, mouse, rabbit and humans) and modern repro set (tests for Reproductive toxicity in adult males, sperm toxicity and reproductive toxicity in females);

(ii) 1984, a subchronic repeated dose toxicity study, similar to OECD TG 408.

We have assessed this information and identified the following issues:

An EOGRTS is a standard information requirement at Annex X that cannot be adapted on the basis of Annex IX, Section 8.7.3., Column 1. In any case, were the information submitted as

[3] ECHA Guidance R.7a, Section R.7.6.2.3.2.

part of a weight of evidence adaptation under Annex XI, Section 2, it is noted that the same information was submitted for such an adaptation for the information requirement for a screening study. It was rejected for that purpose (see Section B.2) and therefore would not a fortiori be valid as a weight of evidence adaptation for an EOGRTS for similar reasons.

Therefore, the information you provided does not fulfil the information requirement.

The specifications for the study design

Premating exposure duration and dose-level setting

The length of premating exposure period must be ten weeks to cover the full spermatogenesis and folliculogenesis before the mating, allowing meaningful assessment of the effects on fertility.

Ten weeks premating exposure duration is required to obtain results adequate for classification and labelling and /or risk assessment. There is no substance specific information in the dossier supporting shorter premating exposure duration.[1]

Therefore, the requested premating exposure duration is at least ten weeks.

In order to be compliant and not to be rejected due to too low dose levels, the highest dose level shall aim to induce systemic toxicity, but not death or severe suffering of the animals, to allow comparison of reproductive toxicity and systemic toxicity. The dose level selection should be based upon the fertility effects. A descending sequence of dose levels should be selected in order to demonstrate any dose-related effect and to establish NOAELs.

If there is no relevant data to be used for dose level setting, it is recommended that range-finding results are reported with the main study.

You have to provide a justification with your study results that demonstrates that the dose level selection meets the conditions described above.

Cohorts 1A and 1B

Cohorts 1A and 1B belong to the basic study design and must be included.

Species and route selection

The study must be performed in rats with oral[4] administration.

Further expansion of the study design

The conditions to include the extension of Cohort 1B are currently not met. Furthermore, no triggers for the inclusion of Cohorts 2A and 2B (developmental neurotoxicity) and Cohort 3 (developmental immunotoxicity) were identified. However, you may expand the study by including the extension of Cohort 1B, Cohorts 2A and 2B and/or Cohort 3 if relevant information becomes available from other studies or during the conduct of this study. Inclusion is justified if the available information meets the criteria and conditions which are described in Column 2, Section 8.7.3., Annex X. You may also expand the study due to other scientific reasons in order to avoid a conduct of a new study. The study design, including any added expansions, must be fully justified and documented. Further detailed guidance on study design and triggers is provided in ECHA Guidance[5].

[4] ECHA Guidance R.7a, Section R.7.6.2.3.2.
[5] ECHA Guidance R.7a, Section R.7.6.

Appendix D: Requirements to fulfil when conducting and reporting new tests for REACH purposes

A. Test methods, GLP requirements and reporting

1. Under Article 13(3) of REACH, all new data generated as a result of this decision must be conducted according to the test methods laid down in a European Commission Regulation or to international test methods recognised by the Commission or ECHA as being appropriate.

2. Under Article 13(4) of REACH, ecotoxicological and toxicological tests and analyses must be carried out according to the GLP principles (Directive 2004/10/EC) or other international standards recognised by the Commission or ECHA.

3. Under Article 10(a)(vi) and (vii) of REACH, all new data generated as a result of this decision must be reported as study summaries, or as robust study summaries, if required under Annex I of REACH. See ECHA Practical Guide on How to report robust study summaries[6].

B. Test material

Before generating new data, you must agree within the joint submission on the chemical composition of the material to be tested (Test Material) which must be relevant for all the registrants of the Substance.

1. Selection of the Test material(s)

   The Test Material used to generate the new data must be selected taking into account the following:
   - the impact of each constituent/ impurity on the test results for the endpoint to be assessed. For example, if a constituent/ impurity of the Substance is known to have an impact on (eco)toxicity, the selected Test Material must contain that constituent/ impurity.

2. Information on the Test Material needed in the updated dossier
   - You must report the composition of the Test Material selected for each study, under the "Test material information" section, for each respective endpoint study record in IUCLID.
   - The reported composition must include all constituents of each Test Material and their concentration values and other parameters relevant for the property to be tested.

This information is needed to assess whether the Test Material is relevant for the Substance.

Technical instructions on how to report the above is available in the manual on How to prepare registration and PPORD dossiers[7].

[6] https://echa.europa.eu/practical-guides
[7] https://echa.europa.eu/manuals

Appendix E: Procedure

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

ECHA followed the procedure detailed in Articles 50 and 51 of REACH.

The compliance check was initiated on 1 October 2020.

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

In your comments on the initial draft decision you requested the deadline to be extended to 42 months. However, you did not provide any proof for the extension need. Please note that the deadline originally proposed in the draft decision already takes sequential testing into account.

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

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

As no amendments were proposed, ECHA adopted the decision under Article 51(3) of REACH.

Appendix F: List of references - ECHA Guidance[8] and other supporting documents

Evaluation of available information
Guidance on information requirements and chemical safety assessment, Chapter R.4 (version 1.1., December 2011), referred to as ECHA Guidance R.4 where relevant.

QSARs, read-across and grouping
Guidance on information requirements and chemical safety assessment, Chapter R.6 (version 1.0, May 2008), referred to as ECHA Guidance R.6 where relevant.

Read-across assessment framework (RAAF, March 2017)[9]

RAAF - considerations on multiconstituent substances and UVCBs (RAAF UVCB, March 2017)[10]

Physical-chemical properties
Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Toxicology
Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7c (version 3.0, June 2017), referred to as ECHA Guidance R.7c in this decision.

Environmental toxicology and fate
Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7b (version 4.0, June 2017), referred to as ECHA Guidance R.7b in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7c (version 3.0, June 2017), referred to as ECHA Guidance R.7c in this decision.

PBT assessment
Guidance on information requirements and chemical safety assessment, Chapter R.11 (version 3.0, June 2017), referred to as ECHA Guidance R.11 in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.16 (version 3.0, February 2016), referred to as ECHA Guidance R.16 in this decision.

Data sharing
Guidance on data-sharing (version 3.1, January 2017), referred to as ECHA Guidance on data sharing in this decision.

OECD Guidance documents[11]

[8] https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment
[9] https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across
[10] https://echa.europa.eu/documents/10162/13630/raaf_uvcb_report_en.pdf/3f79684d-07a5-e439-16c3-d2c8da96a316
[11] http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm

Guidance Document on aqueous–phase aquatic toxicity testing of difficult test chemicals – No 23, referred to as OECD GD 23.

Guidance document on transformation/dissolution of metals and metal compounds in aqueous media – No 29, referred to as OECD GD 29.

Guidance Document on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption – No 150, referred to as OECD GD 150.

Guidance Document supporting OECD test guideline 443 on the extended one-generation reproductive toxicity test – No 151, referred to as OECD GD 151.

Appendix G: Addressees of this decision and their corresponding information requirements

You must provide the information requested in this decision for all REACH Annexes applicable to you.

Registrant Name	Registration number	Highest REACH Annex applicable to you
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]

Where applicable, the name of a third party representative (TPR) may be displayed in the list of recipients whereas ECHA will send the decision to the actual registrant.